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Patent- og
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Improved phytases

Phytases are enzymes that hydrolyze phytate (myo-inositol hexakisphosphate) to myo-inositol and inorganic phospha-te and are known to be valuable feed additives.

Preferred phytases are myo-inositol hexakisphosphate phosphohydrola-ses, such as (myo-inositol hexaphosphate 3-phosphohydrolase, EC 3.1.3.8) and (myo-inositol hexaphosphate 6-phosphohydrolase, EC 3.1.3.26).

A phytase was first described in rice bran in 1907 [Suzuki et al., Bull. Coll. Agr. Tokio Imp. Univ. 7, 495 (1907)] and phytases from Aspergillus species in 1911 [Dox and Golden, J. Biol. Chem. 10, 183-186 (1911)]. Phytases have also been found in wheat bran, plant seeds, animal intestines and in microorganisms [Howsen and Davis, Enzyme Microb. Technol. 5, 377-382 (1983), Lambrechts et al., Biotech. Lett. 14, 61-66 (1992), Shieh and Ware, Appl. Microbiol. 16, 1348-1351 (1968)].

The cloning and expression of the phytase from Aspergillus niger (ficuum) has been described by Van Hartingsveldt et al., in Gene, 127, 87-94 (1993) and in European Patent Application, Publication No. (EP) 420 358 and from Aspergillus niger var. awamori by Piddington et al., in Gene 133, 55-62 (1993).

Cloning, expression and purification of phytases with improved properties have been disclosed in EP 684 313. However, since there is a still ongoing need for further improved phytases, especially with respect to their thermostability, it is an object of the present invention to provide the following process which is, however, not only applicable to phytases.

The present invention relates to improved phytases, viz. phytases of amended characteristics, preferably amended activity characteristics, amended as compared to the phytase(s) it has

been derived from, preferably amended as compared to known phytases. Amended activity characteristics means amended in at least one phytase activity related respect, such as (non-exclusive list): pH stability, temperature stability, pH profile, temperature profile, specific activity (in particular in relation to pH and temperature), substrate specificity, substrate cleavage pattern, substrate binding, position specificity, the velocity and level of release of phosphate from corn, reaction rate, phytate degradation rate), end level of released phosphate reached.

Preferred amended activity characteristics are amended specific activity, preferably increased, and preferably increased at a pH of 3, 4, 5, or 6; amended pH or temperature profile; and/or amended, preferably increased, thermostability, e.g. of an increased melting temperature as measured using DSC.

The present invention also relates to a process for the preparation of a modified protein, wherein in a first step a consensus sequence is determined from a number of highly homologous sequences according to steps a), b) and c) below:

a) at least three, preferably four amino acid sequences are aligned by any standard alignment program known in the art;

b) amino acids at the same position according to such alignment are compared regarding their evolutionary similarity by any standard program known in the art, whereas the degree of similarity provided by such a program which defines the least similarity of the amino acids that is used for the determination of an amino acid of corresponding positions is set to a less stringent number and the parameters are set in such a way that it is possible for the program to determine from only 2 identical amino acids at a corresponding position an amino acid for the consensus protein; however, if among the compared amino

acid sequences are sequences that show a much higher degree of similarity to each other than to the residual sequences, these sequences are represented by their consensus sequence determined as defined in the same way as in the present process for the consensus sequence of the consensus protein or a vote weight of 1 divided by the number of such sequences is assigned to every of -those sequences;

c) in case no common amino acid at a defined position is identified by the program, any of the amino acids, preferably the most frequent amino acid of all such sequences is selected;

in a second step the amino acid sequence of another protein which is homologous to the consensus sequence is compared with the consensus sequence; and

in a third step the consensus sequence or the other protein sequence is modified to define a modified sequence;

the modified sequence is back-translated into a DNA sequence, preferably by using a codon frequency table of the organism in which expression should take place;

the DNA sequence is synthesized by methods known in the art and used either integrated into a suitable expression vector or by itself to transform an appropriate host cell;

the transformed host cell is grown under suitable culture conditions and the other protein is isolated from the host cell or its culture medium by methods known in the art.

In one aspect of the above process, a modified other protein sequence is defined in the third step as follows: Only those amino acid residues are replaced in the amino acid sequence of the other protein which clearly differ from the consensus sequence of this protein family calculated under moderately stringent conditions whereas at all positions of the alignment where no preferred single amino acid can be determined

under moderately stringent conditions the amino acids of the other protein remain unchanged.

In another aspect, the second step comprises determining the active center of the protein, comprising all amino acid residues that are involved in forming the active center, in the consensus sequence, and in the sequence of the other protein as well; and in the third step a modified consensus sequence is defined as follows: Some or all of the amino acids that form the active center of the other protein are inserted in the backbone of the consensus sequence.

In a preferred process, the program used for the comparison of amino acids at a defined position regarding their evolutionary similarity is the program "PRETTY".

Preferably, the active center of the protein is determined by using an analysis of the three-dimensional structure of the protein.

A preferred homologeous protein is an enzyme, a preferred defined protein family is the family of phytases, preferably of fungal origin.

Preferably the amino acid sequence of the phytase is changed by the introduction of at least one mutation selected from the group consisting of

E58A	F54Y
D69K	I73V
D197N	K94A
T214L	R101A
E222T	N153K
E267D	V158I
R291I	A203G
R329H	S205G
S364T	V217A

A379K	A227V
G404A	V234L
	P238A
	Q277E
	A287H
	A292Q
	V366I
	A396S
	E415Q
	G437A
	E451R

whereby the number represents the position in the consensus phytase sequence or a corresponding residue according to an alignment as shown in Fig. 1 when 26 amino acids (signal sequence) are added to the sequences shown in Fig. 1 and the letter before the number represents the amino acid in the phytase which is replaced by the amino acid behind the number.

Preferred host cells are of eukaryotic origin, preferably fungal, such as Aspergillus, or yeast, preferably Saccharomyces or Hansenula.

In another aspect, the invention relates to a modified protein obtainable preferably obtained by any of the above described processes.

In a further aspect, the invention relates to a mutein of the consensus phytase-1, characterized therein that in the amino acid sequence of Figure 2 the following replacements have been effected Q50L, Q50T, Q50G, Q50T-Y51N, Q50L-Y51N or Q50T-K91A.

In a preferred embodiment of this process step b) can also be defined as follows:

b) amino acids at the same position according to such an alignment are compared regarding their evolutionary similarity

by any standard program known in the art, whereas the degree of similarity provided by such program is set at the lowest possible value and the amino acid which is the most similar for at least half of the sequences used for the comparison is selected for the corresponding position in the amino acid sequence of the consensus protein.

By using the above processes the consensus sequence derived from a number of highly homologous sequences can be used in order to replace only certain amino acid residues in the protein in such a manner that only those amino acid residues are replaced which clearly and unambiguously differ from the corresponding consensus sequence of this protein family which has been calculated on moderately stringent conditions. At all other positions of the alignment, however, where the method of the present invention is not able to determine clearly a preferred amino acid residue under moderately stringent conditions the amino acid residues of the other protein are maintained unchanged.

In the alternative, a consensus sequence is determined from homologous sequences as described above. In a second step the active center of the protein comprising all amino acid residues that are involved in forming the active center is determined in the consensus sequence and in the sequence of a single homologous protein as well. The single homologous protein may have preferred properties like high specific activity or different pH dependency of enzymatic activity. In a third step some or all amino acid residues that are involved in forming the active centre of the homologeous protein are inserted into the backbone of the consensus sequence. The result thereof is a chimeric protein having the active centre derived from a single protein and the backbone of the consensus sequence.

The active centre of the protein can be determined e.g. by using any analysis of the three-dimensional structure of the protein, e.g. by homology modelling on the basis of a known 3D-structure of a known protein. Frequently the single homologeous protein is an enzyme.

It is also an object of the present invention to provide a consensus protein obtainable preferably obtained, by such processes and specifically the consensus protein, which has the amino acid sequences shown in Figures 2, 4 and 6 or a variant thereof. A "variant" refers in the context of the present invention to a consensus protein with amino acid sequence shown in Figure 2, 5, 7, and 8 wherein at one or more positions amino acids have been deleted, added or replaced by one or more other amino acids with the proviso that the resulting sequence provides for a protein whose basic properties like enzymatic activity (type of and specific activity), thermostability, activity in a certain pH-range (pH-stability) have not significantly been changed. "Significantly" means in this context that a man skilled in the art would say that the properties of the variant may still be different but would not be unobvious over the ones of the consensus protein with the amino acid sequence of Figure 2 itself.

A "mutein" refers in the context of the present invention to replacements of the amino acid in the amino acid sequences of the consensus proteins shown in Figure 2 which lead to consensus proteins with further improved properties e.g. activity. Such muteins can be defined and prepared on the basis of the teachings given in European Patent Application number 97810175.6, e. g. Q50L, Q50T, Q50G, Q50L-Y51N, or Q50T-Y51N. "Q50L" means in this context that at position 50 of the amino

acid sequence (Figure 2) the amino acid Q has been replaced by amino acid L.

In addition, a food, feed or pharmaceutical composition comprising a consensus protein as defined above is also an object of the present invention.

In this context "at least three preferably four amino acid sequences of such defined protein family" means that three, four, five, six to 12, 20, 50 or even more sequences can be used for the alignment and the comparison to create the amino acid sequence of the consensus protein. "Sequences of a defined protein family" means that such sequences fold into a three dimensional structure, wherein the alpha-helixes, the beta-sheets and beta-turns are at the same position so that such structures are, as called by the man skilled in the art, largely superimposable. Furthermore these sequences characterize proteins which show the same type of biological activity, e.g. a defined enzyme class, e.g. the phytases. As known in the art, the three dimensional structure of one of such sequences is sufficient to allow the modelling of the structure of the other sequences of such a family. An example, how this can be effected, is given in the Reference Example of the present case. "Evolutionary similarity" in the context of the present invention refers to a scheme which classifies amino acids regarding their structural similarity which allows that one amino acid can be replaced by another amino acid with a minimal influence on the overall structure, as this is done e.g. by programs, like "PRETTY", known in the art. The phrase "the degree of similarity provided by such a program...is set to less stringent number" means in the context of the present invention that values for the parameters which determine the degree of similarity in the program used in the practice of the present

invention are chosen in a way to allow the program to define a common amino acid for a maximum of positions of the whole amino acid sequence, e. g. in case of the program PRETTY a value of 2 or 3 for the THRESHOLD and a value of 2 for the PLURALITY can be choosen. Furthermore, "a vote weight of one divided by the number of such sequences" means in the context of the present invention that the sequences which define a group of sequences with a higher degree of similarity as the other sequences used for the determination of the consensus sequence only contribute to such determination with a factor which is equal to one divided by a number of all sequences of this group.

As mentioned before should the program not allow to select the most similar amino acid, the most frequent amino acid is selected, should the latter be impossible the man skilled in the art will select an amino acid from all the sequences used for the comparison which is known in the art for its property to improve the thermostability in proteins as discussed e.g. by Janecek, S. (1993), Process Biochem. 28, 435-445 or Fersht, A. R. & Serrano, L. (1993), Curr. Opin. Struct. Biol. 3, 75-83. Alber, T. (1989), Annu. Rev. Biochem. 58, 765-798 or Matthews, B. W. (1987), Biochemistry 26, 6885-6888. Matthews, B. W. (1991), Curr. Opin. Struct. Biol. 1, 17-21.

The stability of an enzyme is a critical factor for many industrial applications. Therefore, a lot of attempts, more or less successful, have been made to improve the stability, preferably the thermostability of enzymes by rational (van den Burg et al., 1998) or irrational approaches (Akanuma et al., 1998). The forces influencing the thermostability of a protein are the same as those that are responsible for the proper folding of a peptide strand (hydrophobic interactions, van der Waals interactions, H-bonds, salt bridges, conformational strain

(Matthews, 1993). Furthermore, as shown by Matthews et al. (1987), the free energy of the unfolded state has also an influence on the stability of a protein. Enhancing of protein stability means to increase the number and strength of favorable interactions and to decrease the number and strength of unfavorable interactions. It has been possible to introduce disulfide linkages (Sauer et al, 1986) to replace glycine with alanine residues or to increase the proline content in order to reduce the free energy of the unfolded state (Margarit et al, 1992; Matthews, 1987a). Other groups concentrated on the importance of additional H-bonds or salt bridges for the stability of a protein (Blaber et al, 1993) or tried to fill cavities in the protein interior to increase the buried hydrophobic surface area and the van der Waals interactions (Karpusas et al, 19898). Furthermore, the stabilization of secondary structure elements, especially a-helices, for example, by improved helix capping, was also investigated (Munoz & Serrano, 1995).

However, there is no fast and promising strategy to identify amino acid replacements which will increase the stability, preferably the thermal stability of a protein. Commonly, the 3D structure of a protein is required to find locations in the molecule where an amino acid replacement possibly will stabilize the protein's folded state. Alternative ways to circumvent this problem are either to search for a homologous protein in a thermo- or hyperthermophile organism or to detect stability-increasing amino acid replacements by a random mutagenesis approach. This latter possibility succeeds in only 103 to 104 mutations and is restricted to enzymes for which a fast screening procedure is available (Arase et al, 1993; Risse et al, 1992). For all these approaches, success was

variable and unpredictable and, if successful, the thermostability enhancements nearly always were rather small.

Here we present an alternative way to improve the thermostability of a protein. Imanaka et al (1986) were among the first to use the comparisons of homologous proteins to enhance the stability of a protein. They used a comparison of proteases from thermophilic with homologous ones of mesophilic organisms to enhance the stability of a mesophilic protease. Serrano et al (1993) used the comparison of the amino acid sequences of two homologous mesophilic RNases to construct a more thermostable Rnase. They mutated individually all of the residues that differ between the two and combined the mutations that increase the stability in a multiple mutant. Pantoliano et al (1989) and, in particular, Steipe et al (1994) suggested that the most frequent amino acid at every position of an alignment of homologous proteins contribute to the largest amount to the stability of a protein. Steipe et al (1994) proved this for a variable domain of an immunoglobulin, whereas Pantoliano et al (1989) looked for positions in the primary sequence of subtilisin in which the sequence of the enzyme chosen to be improved for higher stability was singularly divergent. Their approach resulted in the replacement M50F which increased the Tm of subtilisin by 1.8 *C.

Steipe et al. (1994) proved on a variable domain of immunoglobulin that it is possible to predict a stabilizing mutation with better than 60% success rate just by using a statistical method which determines the most frequent amino acid residue at a certain position of this domain. It was also suggested that this method would provide useful results not only for stabilization of variable domains of antibodies but also for domains of other proteins. However, it was never mentioned that

this method could be extended to the entire protein. Furthermore, nothing is said about the program which was used to calculate the frequency of amino acid residues at a distinct position or whether scoring matrices were used as in the present case.

It is therefore an object of the present invention to provide a process for the preparation of a consensus protein comprising a process to calculate an amino acid residue for nearly all positions of a so-called consensus protein and to synthesize a complete gene from this sequence that could be expressed in a pro- or eukaryotic expression system.

DNA sequences of the present invention can be constructed starting from genomic or cDNA sequences coding for proteins, e.g. phytases known in the art [for sequence information see references mentioned above, e.g.

EP 684 313 or sequence data bases, for example like Genbank (Intelligenetics, California, USA), European Bioinformatics Institute (Hinston Hall, Cambridge, GB), NBRF (Georgetown University, Medical Centre, Washington DC, USA) and Vecbase (University of Wisconsin, Biotechnology Centre, Madison, Wisconsin, USA) or disclosed in the figures by methods of in vitro mutagenesis [see e.g. Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory Press, New York]. A widely used strategy for "site directed mutagenesis", as originally outlined by Hurchinson and Edgell [J. Virol. 8, 181 (1971)], involves the annealing of a synthetic oligonucleotide carrying the desired nucleotide substitution to a target region of a single-stranded DNA sequence wherein the mutation should be introduced [for review see Smith, Annu. Rev. Genet. 19, 423 (1985) and for improved methods see references 2-6 in Stanssen et al., Nucl. Acid Res., 17, 4441-4454 (1989)]. Another possibility of

mutating a given DNA sequence which is also preferred for the practice of the present invention is the mutagenesis by using the polymerase chain reaction (PCR). DNA as starting material can be isolated by methods known in the art and described e.g. in Sambrook et al. (Molecular Cloning) from the respective strains. For strain information see, e.g. EP 684 313 or any depository authority indicated below. Aspergillus niger [ATCC 9142], Myceliophthora thermophila [ATCC 48102], Talaromyces thermophilus [ATCC 20186] and Aspergillus fumigatus [ATCC 34625] have been redeposited according to the conditions of the Budapest Treaty at the American Type Culture Cell Collection under the following accession numbers: ATCC 74337, ATCC 74340, ATCC 74338 and ATCC 74339, respectively. It is however, understood that DNA encoding a consensus protein in accordance with the present invention can also be prepared in a synthetic manner as described, e.g. in EP 747 483 or the examples by methods known in the art.

The process of the present invention can preferably be used in order to improve the thermostability of the enzyme phytase. After having constructed different consensus phytase sequences it was possible to decide whether single amino acid replacements had a positive or a negative effect on the protein stability. It is therefore another subject of the present invention to improve the thermostability of a phytase.

In this embodiment single amino acids are changed in the sequence of the phytase by the introduction of at least one mutation selected from the group consisting of

E58A	F54Y
D69K	I73V
D197N	K94A
T214L	R101A

E222T	N153K
E267D	V158I
R291I	A203G
R329H	S205G
S364T	V217A
A379K	A227V
G404A	V234L
	P238A
	Q277E
	A287H
	A292Q
	V366I
	A396S
	E415Q
	G437A
	E451R

In the above-given mutations the number represents the position in the consensus phytase-1-sequence as given in Figure 2 and the letter before the number represents the amino acid in the phytase which is replaced by the respective amino acid behind the number. The numbers given correspond to the consensus phytase sequence or relate to a corresponding residue calculated by an alignment as shown in Figure 1 when 26 amino acids (signal sequence) are added to the sequences shown in Fig. 1. Those mutations can be introduced into consensus sequences or into sequences of specific enzymes which have been improved by a process of the present invention. The above-mentioned amino acid replacements have a positive effect on the protein stability.

Once complete DNA sequences of the present invention have been obtained they can be integrated into vectors by methods

known in the art and described e.g. in Sambrook et al. (s.a.) to overexpress the encoded polypeptide in appropriate host systems. However, a man skilled in the art knows that also the DNA sequences themselves can be used to transform the suitable host systems of the invention to get overexpression of the encoded polypeptide. Appropriate host systems are for example fungi, like Aspergilli, e.g. Aspergillus niger [ATCC 9142] or Aspergillus ficuum [NRRL 3135] or like Trichoderma, e.g. Trichoderma reesei or yeasts, like Saccharomyces, e.g. Saccharomyces cerevisiae or Pichia, like Pichia pastoris, or Hansenula polymorpha, e.g. H. polymorpha (DSM5215) or plants, as described, e.g. by Pen et al., Bio/Technology 11, 811-814 (1994). A man skilled in the art knows that such microorganisms are available from depository authorities, e.g. the American Type Culture Collection (ATCC), the Centraalbureau voor Schimmelcultures (CBS) or the Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH (DSM) or any other depository authority as listed in the Journal "Industrial Property" [(1991) 1, pages 29-40]. Bacteria which can be used are e.g. E. coli, Bacilli as, e.g. Bacillus subtilis or Streptomyces, e.g. Streptomyces lividans (see e.g. Anné and Mallaert in FEMS Microbiol. Letters 114, 121 (1993). E. coli, which could be used are E. coli K12 strains e.g. M15 [described as DZ 291 by Villarejo et al. in J. Bacteriol. 120, 466-474 (1974)], HB 101 [ATCC No. 33694] or E. coli SG13009 [Gottesman et al., J. Bacteriol. 148, 265-273 (1981)].

Vectors which can be used for expression in fungi are known in the art and described e.g. in EP 420 358, or by Cullen et al. [Bio/Technology 5, 369-376 (1987)] or Ward in Molecular Industrial Mycology, Systems and Applications for Filamentous Fungi, Marcel Dekker, New York (1991), Upshall et al.

[Bio/Technology 5, 1301-1304 (1987)] Gwynne et al. [Bio/Technology 5, 71-79 (1987)], Punt et al. [J. Biotechnol. 17, 19-34 (1991)] and for yeast by Sreekrishna et al. [J. Basic Microbiol. 28, 265-278 (1988), Biochemistry 28, 4117-4125 (1989)], Hitzemann et al. [Nature 293, 717-722 (1981)] or in EP 183 070, EP 183 071, EP 248 227, EP 263 311. Suitable vectors which can be used for expression in E. coli are mentioned, e.g. by Sambrook et al. [s.a.] or by Fiers et al. in Procd. 8th Int. Biotechnology Symposium" [Soc. Franc. de Microbiol., Paris (Durand et al., eds.), pp. 680-697 (1988)] or by Bujard et al. in Methods in Enzymology, eds. Wu and Grossmann, Academic Press, Inc. Vol. 155, 416-433 (1987) and Stüber et al. in Immunological Methods, eds. Lefkovits and Pernis, Academic Press, Inc., Vol. IV, 121-152 (1990). Vectors which could be used for expression in Bacilli are known in the art and described, e.g. in EP 405 370, Procd. Natl. Acad. Sci. USA 81, 439 (1984) by Yansura and Henner, Meth. Enzymol. 185, 199-228 (1990) or EP 207 459. Vectors which can be used for the expression in H. Polymorpha are known in the art and described, e.g. in Gellissen et al., Biotechnology 9, 291-295 (1991).

Either such vectors already carry regulatory elements, e.g. promotors, or the DNA sequences of the present invention can be engineered to contain such elements. Suitable promotor elements which can be used are known in the art and are, e.g. for Trichoderma reesei the cbh1- [Haarki et al., Biotechnology 7, 596-600 (1989)] or the pki1-promotor [Schindler et al., Gene 130, 271-275 (1993)], for Aspergillus oryzae the amy-promotor [Christensen et al., Abstr. 19th Lunteren Lectures on Molecular Genetics F23 (1987), Christensen et al., Biotechnology 6, 1419-1422 (1988), Tada et al., Mol. Gen. Genet. 229, 301 (1991)], for Aspergillus niger the glaA- [Cullen et al., Bio/Technology 5,

369-376 (1987), Gwynne et al., Bio/Technology 5, 713-719 (1987), Ward in Molecular Industrial Mycology, Systems and Applications for Filamentous Fungi, Marcel Dekker, New York, 83-106 (1991)], alcA- [Gwynne et al., Bio/Technology 5, 718-719 (1987)], suc1- [Boddy et al., Curr. Genet. 24, 60-66 (1993)], aphA- [MacRae et al., Gene 71, 339-348 (1988), MacRae et al., Gene 132, 193-198 (1993)], tpiA- [McKnight et al., Cell 46, 143-147 (1986), Upshall et al., Bio/Technology 5, 1301-1304 (1987)], gpdA- [Punt et al., Gene 69, 49-57 (1988), Punt et al., J. Biotechnol. 17, 19-37 (1991)] and the pkiA-promotor [de Graaff et al., Curr. Genet. 22, 21-27 (1992)]. Suitable promotor elements which could be used for expression in yeast are known in the art and are, e.g. the pho5-promotor [Vogel et al., Mol. Cell. Biol., 2050-2057 (1989); Rudolf and Hinnen, Proc. Natl. Acad. Sci. 84, 1340-1344 (1987)] or the gap-promotor for expression in Saccharomyces cerevisiae and for Pichia pastoris, e.g. the aox1-promotor [Koutz et al., Yeast 5, 167-177 (1989); Sreekrishna et al., J. Basic Microbiol. 28, 265-278 (1988)], or the FMD promoter [Hollenberg et al., EPA No. 0299108] or MOX-promotor [Ledeboer et al., Nucleic Acids Res. 13, 3063-3082 (1985)] for H. polymorpha.

Accordingly vectors comprising DNA sequences of the present invention, preferably for the expression of said DNA sequences in bacteria or a fungal or a yeast host and such transformed bacteria or fungal or yeast hosts are also an object of the present invention.

It is also an object of the present invention to provide a system which allows for high expression of proteins, preferably phytases like the consensus phytase of the present invention in Hansenula characterized therein that the codons of the encoding DNA sequence of such a protein have been selected on the basis

of a codon frequency table of the organism used for expression, e.g. yeast as in the present case (see e.g. in Example 3) and optionally the codons for the signal sequence have been selected in a manner as described for the specific case in Example 3. That means that a codon frequency table is prepared on the basis of the codons used in the DNA sequences which encode the amino acid sequences of the defined protein family. Then the codons for the design of the DNA sequence of the signal sequence are selected from a codon frequency table of the host cell used for expression whereby always codons of comparable frequency in both tables are used.

Once such DNA sequences have been expressed in an appropriate host cell in a suitable medium the encoded protein can be isolated either from the medium in the case the protein is secreted into the medium or from the host organism in case such protein is present intracellularly by methods known in the art of protein purification or described in case of a phytase, e.g. in EP 420 358. Accordingly a process for the preparation of a polypeptide of the present invention characterized in that transformed bacteria or a host cell as described above is cultured under suitable culture conditions and the polypeptide is recovered therefrom and a polypeptide when produced by such a process or a polypeptide encoded by a DNA sequence of the present invention are also an object of the present invention.

Once obtained the polypeptides of the present invention can be characterized regarding their properties which make them useful in agriculture any assay known in the art and described e.g. by Simons et al. [Br. J. Nutr. 64, 525-540 (1990)], Schöner et al. [J. Anim. Physiol. a. Anim. Nutr. 66, 248-255 (1991)], Vogt [Arch. Geflügelk. 56, 93-98 (1992)], Jongbloed et al. [J. Anim. Sci., 70, 1159-1168 (1992)], Perney et al. [Poultry Sci.

72, 2106-2114 (1993)], Farrell et al., [J. Anim. Physiol. a. Anim. Nutr. 69, 278-283 (1993), Broz et al., [Br. Poultry Sci. 35, 273-280 (1994)] and Düngelhoef et al. [Animal Feed Sci. Technol. 49, 1-10 (1994)] can be used.

In general the polypeptides of the present invention can be used without being limited to a specific field of application, e.g. in case of phytases for the conversion of inositol polyphosphates, like phytate to inositol and inorganic phosphate.

Furthermore the polypeptides of the present invention can be used in a process for the preparation of a pharmaceutical composition or compound food or feeds wherein the components of such a composition are mixed with one or more polypeptides of the present invention. Accordingly compound food or feeds or pharmaceutical compositions comprising one or more polypeptides of the present invention are also an object of the present invention. A man skilled in the art is familiar with their process of preparation. Such pharmaceutical compositions or compound foods or feeds can further comprise additives or components generally used for such purpose and known in the state of the art.

It is furthermore an object of the present invention to provide a process for the reduction of levels of phytate in animal manure characterized in that an animal is fed such a feed composition in an amount effective in converting phytate contained in the feedstuff to inositol and inorganic phosphate.

In the present context, a phytase is an enzyme or polypeptide that has phytase activity. The phytase is preferably purified, viz. at least 85%, preferably at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% pure. The phytase is preferably isolated. Phytase activity can be determined using

any phytase assay known in the art. A preferred assay is the so-called standard assay herein (see Example 9). A preferred assay temperature is the optimum temperature of the actual phytase, and a preferred assay pH is the optimum pH of the actual phytase. A preferred assay is described in Example 9 herein. Another preferred assay is the FYT assay of example 15 of WO 98/28409, hereby incorporated by reference.

In preferred embodiments, the assay temperature is selected within the range of 20-90°C, more preferably 30-80°C, still more preferably 35-75°C. Preferred assay temperatures are 37°C, 50°C, 60°C, and 70°C.

In further preferred embodiments, the assay pH is selected within the range of pH 2-9, more preferably 3-8, still more preferably 3-6. Preferred assay pH values are 3, 4, 5, 6 and 7.

Amino acid sequence homology (or polypeptide or amino acid homology) is determined as the degree of identity between two sequences. This may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711), see also Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453, hereby incorporated by reference. In release 9.1, for comparing polypeptide sequences, the Length Weight is set to 0, and the Gap Weight is set to 3.0.

The degree of identity or homology between two DNA (nucleic acid) sequences may be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711), see also Needleman, S.B. and Wunsch, C.D., (1970),

Journal of Molecular Biology, 48, 443-453, hereby incorporated by reference. In release 9.1, GAP is used with the following settings for DNA sequence comparison: GAP creation penalty of 50 and GAP extension penalty of 3.

Suitable experimental conditions for determining whether a given DNA or RNA sequence hybridizes to a specified nucleotide or oligonucleotide probe involves presoaking of the filter containing the DNA or RNA fragments to examine for hybridization in 5 x SSC (Sodium chloride/Sodium citrate), (J. Sambrook, E.F. Fritsch, and T. Maniatis, 1989, Molecular Cloning, A Laboratory Manual, 2d edition, Cold Spring Harbor, New York) for 10 min, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution (Sambrook et al. 1989), 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a concentration of 10 ng/ml of a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) Anal. Biochem. 132:6-13), 32P-dCTP-labeled (specific activity > 1 x 109 cpm/µg) probe for 12 hours at approximately 45°C.

The filter is then washed twice for 30 minutes in 2 x SSC, 0.5 % SDS at at least 55°C (low stringency), at at least 60°C (medium stringency), at at least 65°C (medium/high stringency), at at least 70°C (high stringency), or at at least 75°C (very high stringency).

Molecules to which the oligonucleotide probe hybridizes under these conditions are detected using an x-ray film.

Before describing the present invention in more detail a short explanation of the Figures enclosed is given below.

Figure 1: Design of the consensus phytase sequence. The letters represent the amino acid residues in the one-letter code. The following sequences were used for the alignment: phyA from Aspergillus terreus 9A-1 (Mitchell et al, 1997; from amino acid (aa) 27), phyA from A. terreus cbs116.46; (van Loon et al., 1998; from aa 27), phyA from Aspergillus niger var. awamori (Piddington et al, 1993; from aa 27), phyA from A. niger T213; from aa 27), phyA from A. niger strain NRRL3135 (van Hartingsveldt et al, 1993; from aa 27), phyA from Aspergillus fumigatus ATCC 13073 (Pasamontes et al, 1993; from aa 25), phyA from A. fumigatus ATCC 32722 (van Loon et al, 1998; from aa 27), phyA from A. fumigatus ATCC 58128 (van Loon et al., 1998; from aa 27), phyA from A. fumigatus ATCC 26906 (van Loon et al, 1998; from aa 27), phyA from A. fumigatus ATCC 32239 (van Loon et al, 1998; from aa 30), phyA from Emericella nidulans (Pasamontes et al, 1997a; from aa 25), phyA from Talaromyces thermophilus (Pasamontes et al, 1997a; from aa 24), and phyA from Myceliophthora thermophila (Mitchell et al, 1997; from aa 19). The alignment was calculated using the program PILEUP. The location of the gaps was refined by hand. Capitalized amino acid residues in the alignment at a given position belong to the amino acid coalition that establish the consensus residue. In bold, beneath the calculated consensus sequence, the amino acid sequence of the finally constructed consensus phytase (Fcp) is shown. The gaps in the calculated consensus sequence were filled by hand according to principals stated in Example 1.

Figure 2: DNA sequence of the consensus phytase-1 gene (fcp) and of the primers used for the gene construction. The calculated amino acid sequence (Figure 1) was converted into a DNA sequence using the program BACKTRANSLATE (Devereux et al.,

1984) and the codon frequency table of highly expressed yeast genes (GCG program package, 9.0). The signal peptide of the phytase from A. terreus cbs.116.46 was fused to the N-terminus. The bold bases represent the sequences of the oligonucleotides used to generate the gene. The names of the respective oligonucleotides are alternately noted above or below the sequence. The underlined bases represent the start and stop codon of the gene. The bases written in italics show the two introduced Eco RI sites.

Figure 3: Alignment and consensus sequence of five Basidiomycetes phytases. The letters represent the amino acid residues in the one-letter code. The amino acid sequences of the phytases from Paxillus involutus, phyA1 (aa 21) and phyA2 (aa 21, WO 98/28409), Trametes pubescens (aa 24, WO 98/28409), Agrocybe pediades (aa 19, WO 98/28409), and Peniophora lycii (aa 21, WO 98/28409) starting with the amino acid residues mentioned in parentheses, were used for the alignment and the calculation of the corresponding consensus sequence called "Basidio" (Example 2). The alignment was performed by the program PILEPUP. The location of the gaps was refined by hand. The consensus sequence was calculated by the program PRETTY. While a vote weight of 0.5 was assigned to the two P. involutus phytases, all other genes were used with a vote weight of 1.0 for the consensus sequence calculation. At positions, where the program was not able to determine a consensus residues, the Basidio sequence contains a dash. Capitalized amino acid residues in the alignment at a given position belong to the amino acid coalition that establish the consensus residue.

Figure 4: Design of consensus phytase-10 amino acid sequence. Adding the phytase sequence of Thermomyces lanuginosa (Berka et al., 1998) and the consensus sequence of the phytases from five Basidiomycetes to the alignment of Figure 1, an improved consensus sequence was calculated by the program PRETTY. Additionally, the amino acid sequence of A. niger T213 was omitted, therefore, using a vote weight of 0.5 for the remaining A. niger phytase sequences. For further information see Example 2.

Figure 5: DNA and amino acid sequence of consensus phytase-10. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The sequence of the oligonucleotides which were used to assemble the gene are in bold letters. The label of oligonucleotides and the amino acids, which were changed compared to those for consensus phytase-1, are underlined and their corresponding triplets are highlighted in small cases. The fcp10 gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6, CP-7.10, CP-8.10, CP-9.10, CP-10.10, CP-11.10, CP-12.10, CP-13.10, CP-14.10, CP-15.10, CP-16.10, CP-17.10, CP18.10, CP-19.10, CP-20.10, CP-21.10, CP-22.10. The newly synthesized oligonucleotides are additionally marked by number 10. The phytase contains the following 32 exchanges: Y54F, E58A, D69K, D70G, A94K, N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V, A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V, R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E. The mutations accentuated in bold letters revealed a stabilizing effect on consensus phytase-1 as tested as single mutation in consensus phytase-1.

Figure 6: Alignment for the design of consensus phytase-11. In contrast to the design of consensus phytase-10, for the design of the amino acid sequence of consensus phytase-11, all Basidiomycetes phytases were used as independent sequences using an assigned vote weight of 0.2 for each Basidiomycetes sequence. Additionally, the amino acid sequence of A. niger T213 was used in that alignment, again.

Figure 7: DNA and amino acid sequence of consensus phytase-1-thermo[8]-Q50T-K91A. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues are underlined. The stop codon of the gene is marked by a star (*).

Figure 8: DNA and amino acid sequence of consensus phytase-10-thermo[3]-Q50T-K91A. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues are underlined. The stop codon of the gene is marked by a star (*).

Figure 9: DNA and amino acid sequence of A. fumigatus ATCC 13073 phytase alpha-mutant. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues are underlined. The stop codon of the gene is marked by a star (*).

Figure 10: DNA and amino acid sequence of consensus phytase-7. The amino acids are written above the corresponding DNA sequence using the one-letter code. The sequence of the oligonucleotides used to assemble the gene are in bold letters.

Oligonucleotides and amino acids that were exchanged are underlined and their corresponding triplets are highlighted in small cases. The fcp7 gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3, CP-4.7, CP-5.7, CP-6, CP-7, CP-8.7, CP-9, CP-10.7, CP-11.7, CP-12.7, CP-13.7, CP-14.7, CP-15.7, CP-16, CP-17.7, CP-18.7, CP-19.7, CP-20, CP-21, CP-22. The newly synthesized oligonucleotides are additionally marked by number 7. The phytase contains the following 24 exchanges in comparison to the original consensus phytase: S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S.

Figure 11: Differential scanning calorimetry (DSC) of consensus phytase-1 and consensus phytase-10. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-10 (upper graph) yielded a melting temperature of 85.4°C, which is 7.3°C higher than the melting point of consensus phytase-1 (78.1°C, lower graph).

Figure 12: Differential scanning calorimetry (DSC) of consensus phytase-10-thermo-Q50T and consensus phytase-10-thermo-Q50T-K91A. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-10-thermo-Q50T (upper graph) yielded a melting temperature of 88.6°C, while the melting point of consensus phytase-10-thermo-Q50T-K91A was found at 89.3°C.

Figure 13: Comparison of the temperature optimum between consensus phytase-1, consensus phytase-10 and consensus phytase-10-thermo-Q50T. For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 37 and 86°C. The diluted supernatant of transformed S. cerevisiae strains was used for the determination. The other components of the supernatant showed no influence on the determination of the temperature optimum: ∧, consensus phytase-1; ◇, consensus phytase-10; ■, consensus phytase 10-thermo-Q50T.

Figure 14: pH-dependent activity profile and substrate specificity of consensus phytase-10 and its variants thermo-Q50T and thermo-Q50T-K91A. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows the pH-dependent activity profile of consensus phytase-10 (□), consensus phytase-10-thermo-Q50T (•), and consensus phytase-10-thermo-Q50T-K91A (∧). Graph b) shows the corresponding substrate specificity tested by replacement of phytate by the indicated compounds in the standard assay; open bars, consensus phytase-10 (grey bars, consensus phytase-10-thermo-Q50T; dark bars, consensus phytase-10-thermo-Q50T-K91A). The numbers correspond to the following compounds: 1, phytate; 2, p-nitrophenyl phosphate; 3, phenyl phosphate; 4, fructose-1,6-bisphosphate; 5, fructose-6-phosphate; 6, glucose-6-phosphate; 7, ribose-5-phosphate; 8, DL-glycerol-3-phosphate; 9, glycerol-2-phosphate; 10, 3-phosphoglycerate; 11, phosphoenolpyruvate; 12, AMP; 13, ADP; 14, ATP.

Figure 15: pH-dependent activity profile and substrate specificity of consensus phytase-1-thermo[8]-Q50T and of consensus phytase-1-thermo[8]-Q50T-K91A. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows the pH-dependent activity profile of the Q50T- (■) and the Q50T-K91A-variant (•). Graph b) shows the corresponding substrate specificities tested by replacement of phytate by the indicated compounds in the standard assay (open bars, consensus phytase-1-thermo[8]-Q50T; filled bars, consensus phytase-1-thermo[8]-Q50T-K91A). The substrates are listed in the legend of Figure 14.

Figure 16: Differential scanning calorimetry (DSC) of consensus phytase-1-thermo[8]-Q50T and consensus phytase-1-thermo[8]-Q50T-K91A. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-1-thermo[8]-Q50T (upper graph) showed a melting temperature of 84.7°C, while the melting point of consensus phytase-1-thermo[8]-Q50T-K91A was found at 85.7°C.

Figure 17: Comparison of the temperature optimum between consensus phytase-1, consensus phytase-1-thermo[3] and consensus phytase-1-thermo[8]. For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 37 and 86°C. Purified protein from the supernatant of transformed S. cerevisiae strains was used for the determination. O, consensus phytase-1; □, consensus phytase-1-thermo[3]; ▲, consensus phytase 1-thermo[8].

<u>Figure 18</u>: Comparison of the pH-dependent activity profile and substrate specificity of consensus phytase-1, consensus phytase-7, and of the phytase from A. niger NRRL 3135. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows the pH-dependent activity profile of consensus phytase-1 (■), the phytase from A. niger NRRL 3135 (O), and of consensus phytase-7 (▲). Graph b) shows the corresponding substrate specificity tested by replacement of phytate by the indicated compounds in the standard assay (black bars, A. niger NRRL 3135 phytase; grey bars, consensus phytase-1, dashed bars, consensus phytase-7). The substrates are listed in the legend of Figure 14.

<u>Figure 19</u>: Differential scanning calorimetry (DSC) of the phytase from A. fumigatus ATCC 13073 and of its stabilized alpha-mutant, which contains the following amino acid exchanges F55Y, V100I, F114Y, A243L, S265P, N294D.

The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus A. fumigatus 13073 phytase (upper graph) revealed a melting temperature of 62.5°C, while the melting point of the alpha-mutant was found at 67.0°C.

<u>Figure 20</u>: Comparison of the temperature optimum of A. fumigatus 13073 wild-type, its A. fumigatus alpha-mutant, and a further stabilized alpha-mutant (E59A-S154N-R329H-S364T-G404A). For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between

37 and 75°C. The diluted supernatant of transformed S. cerevisiae strains was used for the determination. The other components of the supernatant showed no influence on the determination of the temperature optimum. O, A. fumigatus ATCC 13073 phytase; ▲, A. fumigatus ATCC 13073 alpha-mutant; □, A. fumigatus ATCC 13073 alpha-mutant-(E59A-S154N-R329H-S364T-G404A)-Q27T; ■, A. fumigatus ATCC 13073 alpha-mutant-(E59A-S154N-R329H-S364T-G404A)-Q27T-K68A. Q27T and K68A corresponds to consensus phytase-1 Q50T and K91A, respectively.

Figure 21: Amino acid sequence of consensus phytase 12 (consphy12) which contains a number of active site residues transferred from the "basidio" consensus sequence to consensus phytase-10-thermo-Q50T-K91A.

Example 1

Consensus phytase-1

The amino acid sequence of consensus phytase-1 (fungal consensus phytase, fcp) was designed and calculated as described in Examples 1-2 of EP 0897985. Table 1 below shows the origin and vote weight of the phytase amino acid sequences used for the design of consensus phytase-1. The consensus phytase-1 sequence was furthermore converted into a DNA sequence as described in Example 3 of EP 0897985, and the consensus phytase-1 gene was constructed and cloned as described in Example 4 of EP 0897985. EP 0897985 is hereby incorporated by reference.

Table 1

Origin and vote weight of the phytase amino acid sequences

- phyA from Aspergillus terreus 9A-1, aa 27, vote weight 0.5 (Mitchell et al., 1997)

- phyA from Aspergillus terreus cbs116.46, aa 27, vote weight 0.5 (van Loon et al., 1998)
- phyA from Aspergillus niger var. awamori, aa 27, vote weight 0.33 (Piddington et al., 1993)
- phyA from Aspergillus niger T213, aa 27, vote weight 0.33
- phyA from Aspergillus niger strain NRRL3135, aa 27, vote weight 0.33 (van Hartingsveldt et al., 1993)
- phyA from Aspergillus fumigatus ATCC 13073, aa 26, vote weight 0.2 (Pasamontes et al., 1997)
- phyA from Aspergillus fumigatus ATCC 32722, aa 26, vote weight 0.2 (van Loon et al., 1998)
- phyA from Aspergillus fumigatus ATCC 58128, aa 26, vote weight 0.2 (van Loon et al., 1998)
- phyA from Aspergillus fumigatus ATCC 26906, aa 26, vote weight 0.2 (van Loon et al., 1998)
- phyA from Aspergillus fumigatus ATCC 32239, aa 30, vote weight 0.2 (van Loon et al., 1998)
- phyA from Emericella nidulans , aa 25, vote weight 1.0 (Roche Nr. R1288, Pasamontes et al., 1997a)
- phyA from Talaromyces thermophilus ATCC 20186, aa 24, vote weight 1.0 (Pasamontes et al., 1997a)
- phyA from Myceliophthora thermophila, aa 19, vote weight 1.0 (Mitchell et al., 1997)

Example 2

Design of an improved consensus phytase (consensus phytase-10) amino acid sequence

The alignments used for the design of consensus phytase-10 were calculated using the program PILEUP from the Sequence Analysis Package Release 9.0 (Devereux et al., 1984) with the

standard parameter (gap creation penalty 12, gap extension penalty 4). The location of the gaps was refined using a text editor.

The following sequences were used for the alignment of the Basiodiomycetes phytases starting with the amino acid (aa) mentioned in Table 2:

Table 2

Origin and vote weight of five Basidiomycetes phytases used for the calculation of the corresponding amino acid consensus sequence (basidio)

- phyA1 from Paxillus involutus NN005693, aa 21, vote weight 0.5 (WO 98/28409)
- phyA2 from Paxillus involutus NN005693, aa 21, vote weight 0.5 (WO 98/28409)
- phyA from Trametes pubescens NN9343, aa 24, vote weight 1.0 (WO 98/28409)
- phyA from Agrocybe pediades NN009289, aa 19, vote weight 1.0 (WO 98/28409)
- phyA from Peniophora lycii NN006113, aa 21, vote weight 1.0 (WO 98/28409)

The alignment is shown in Figure 3.

In Table 3 the genes, which were used for the performance of the final alignment, are arranged. The first amino acid (aa) of the sequence which is used in the alignment is mentioned behind the organism designation.

Table 3

Origin and vote weight of the phytase sequences used for the design of consensus phytase 10

- phyA from Aspergillus terreus 9A-1, aa 27, vote weight 0.5 (Mitchell et al., 1997)
- phyA from Aspergillus terreus cbs116.46, aa 27, vote weight 0.5 (van Loon et al., 1998)
- phyA from Aspergillus niger var. awamori, aa 27, vote weight 0.5 (Piddington et al., 1993)
- phyA from Aspergillus niger strain NRRL3135, aa 27, vote weight 0.5 (van Hartingsveldt et al., 1993)
- phyA from Aspergillus fumigatus ATCC 13073, aa 26, vote weight 0.2 (Pasamontes et al., 1997)
- phyA from Aspergillus fumigatus ATCC 32722, aa 26, vote weight 0.2 (van Loon et al., 1998)
- phyA from Aspergillus fumigatus ATCC 58128, aa 26, vote weight 0.2 (van Loon et al., 1998)
- phyA from Aspergillus fumigatus ATCC 26906, aa 26, vote weight 0.2 (van Loon et al., 1998)
- phyA from Aspergillus fumigatus ATCC 32239, aa 30, vote weight 0.2 (van Loon et al., 1998)
- phyA from Emericella nidulans , aa 25, vote weight 1.0 (Roche Nr. R1288, Pasamontes et al., 1997a)
- phyA from Talaromyces thermophilus ATCC 20186, aa 24, vote weight 1.0 (Pasamontes et al., 1997a)
- phyA from Myceliophthora thermophila, aa 19, vote weight 1.0 (Mitchell et al., 1997)
- phyA from Thermomyces lanuginosa, aa 36, vote weight 1.0 (Berka et al., 1998)
- Consensus sequence of five Basidiomycetes phytases, vote weight 1.0 (Basidio, Figure 3)

The corresponding alignment is shown in Figure 4.

Calculation of the amino acid sequence of consensus-10

To improve the alignment, we added the original consensus sequence of five phytases from four different Basidiomycetes, called Basidio, still containing the undefined sequence positions (see Figure 3), nearly all phytase sequences used for calculation of the original consensus phytase and one new phytase sequence from the Ascomycete Thermomyces lanuginosa to a larger alignment. Using the consensus sequence of the basidiomycetal phytase sequences, does not pay regard to the diversity among the five amino acid sequences, but pays regard to the common and different amino acid residues between the phytases from the Ascomycetes and the Basidiomycetes.

We set plurality on 2.0 and threshold on 3. The used vote weight are listed in Table 3. The alignment and the corresponding consensus sequence is presented in Figure 4. The new consensus phytase sequence has 32 different amino acids in comparison to the original consensus phytase. Positions for which the program PRETTY was not able to calculate a consensus amino acid residue were filled according to rules mentioned in Example 1. None of the residues suggested by the program was replaced.

Furthermore, we included all Basidiomycetes phytases as single amino acid sequences but assigning a vote weight of 0.2 in the alignment. The corresponding alignment is shown in Figure 6. The calculated consensus amino acid sequence (consensus phytase-11) has the following differences to the sequence of consensus phytase-10. Letter X means that the program was not able to calculate a consensus amino acid; the amino acid in

parenthesis corresponds to the amino acid finally included into the consensus phytase-10.

D35X, X(K)69K, X(E)100E, A101R, Q134N, X(K)153N, X(H)190H, X(A)204S, X(E)220D, E222T, V227A, X(R)271R, H287A, X(D)288D, X(K)379K, X(I)389I, E390X, X(E)415E, X(A)416A, X(R)446L, E463A, whereas the numbering is as in Fig. 5.

We also checked single amino acid replacements suggested by the improved consensus sequences 10 and 11 on their influence on the stability of the original consensus phytase. The approach is described in example 3.

Conversion of consensus phytase-10 amino acid sequence to a DNA sequence

The first 26 amino acid residues of A. terreus cbs116.46 phytase were used as signal peptide and, therefore, fused to the N-terminus of consensus phytase-10. The used procedure is further described in Example 1.

The resulting sequence of the fcp10 gene is shown in Figure 5.

Construction and cloning of the consensus phytase-10 gene (fcp10)

The calculated DNA sequence of fcp10 was divided into oligonucleotides of 85 bp, alternately using the sequence of the sense and the anti-sense strand. Every oligonucleotide overlaps 20 bp with its previous and its following oligonucleotide of the opposite strand. The location of all primers, purchased by Microsynth, Balgach (Switzerland) and obtained in a PAGE-purified form, is indicated in Figure 5.

PCR-Reactions

In three PCR reactions, the synthesized oligonucleotides were composed to the entire gene. For the PCR, the High Fidelity Kit from Boehringer Mannheim (Boehringer Mannheim, Mannheim, Germany) and the thermo cycler The ProtokolTM from AMS Biotechnology (Europe) Ltd. (Lugano, Switzerland) was used. The following oligonucleotides were used in a concentration of 0.2 pMol/ml.

Mix 1.10: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6, CP-7.10, CP-8.10, CP-9.10, CP-10.10

Mix 2.10: CP-9.10, CP-11.10, CP-12.10, CP-13.10, CP-14.10, CP-15.10, CP-16.10, CP-17.10, CP18.10, CP-19.10, CP-20.10, CP-21.10, CP-22.10

The newly synthesized oligonucleotides are marked by number 10. The phytase contains the following 32 exchanges, which are underlined in Figure 5, in comparison to the original consensus phytase: Y54F, E58A, D69K, D70G, A94K, N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V, A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V, R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E.

Four short PCR primer were used for the assembling of the oligonucleotides:

CP-a: *Eco RI*

5'-TATAT*GAATTC*ATGGGCGTGTTCGTC-3'

CP-b:

5'-TGAAAAGTTCATTGAAGGTTTC-3'

CP-c.10:

5'-TCTTCGAAAGCAGTACACAAAC-3'

CP-e: *Eco RI*

5'-TATAT*GAATTC*TTAAGCGAAAC-3'

PCR reaction a: 10 µl Mix 1.10 (2.0 pmol of each oligonucleotide)
2 µl nucleotides (10 mM each nucleotide)
2 µl primer CP-a (10 pmol/ml)
2 µl primer CP-c.10 (10 pmol/ml)
10,0 µl PCR buffer
0.75 µl polymerase mixture
73.25 µl H_2O

PCR reaction b: 10 µl Mix 2.10 (2.0 pmol of each oligonucleotide)
2 µl nucleotides (10 mM each nucleotide)
2 µl primer CP-b (10 pmol/ml)
2 µl primer CP-e (10 pmol/ml)
10,0 µl PCR buffer
0.75 µl polymerase mixture (2.6 U)
73.25 µl H_2O

Reaction conditions for PCR reaction a and b:

step 1	2 min - 45°C
step 2	30 sec - 72°C
step 3	30 sec - 94°C
step 4	30 sec - 52°C
step 5	1 min - 72°C

Step 3 to 5 were repeated 40-times.

The PCR products (670 and 905 bp) were purified by an agarose gel electrophoresis (0.9% agarose) and a following gel extraction (QIAEX II Gel Extraction Kit, Qiagen, Hilden, Germany). The purified DNA fragments were used for the PCR reaction c.

PCR reaction c:	6 µl PCR product of reaction a ≈50 ng)
	6 µl PCR product of reaction b ≈50 ng)
	2 µl primer CP-a (10 pmol/ml)
	2 µl primer CP-e (10 pmol/ml)
	10,0 µl PCR buffer
	0.75 µl polymerase mixture (2.6 U)
	73.25 µl H_2O

Reaction conditions for PCR reaction c:

step 1	2 min - 94°C
step 2	30 sec - 94°C
step 3	30 sec - 55°C
step 4	1 min - 72°C

Step 2 to 4 were repeated 31-times.

The resulting PCR product (1.4 kb) was purified as mentioned above, digested with Eco RI, and ligated in an Eco RI-digested and dephosphorylated pBsk(-)-vector (Stratagene, La Jolla, CA, USA). 1 µl of the ligation mixture was used to transform E. coli XL-1 competent cells (Stratagene, La Jolla, CA, USA). All standard procedures were carried out as described by Sambrook et al. (1987). The DNA sequence of the constructed gene (fcp10) was checked by sequencing as known in the art.

Example 3

Increasing the thermostability of consensus phytase-1 by introduction of single mutations suggested by the amino acid sequence of consensus phytase-10 and consensus phytase-11

In order to increase the thermostability of homologous genes, it is also possible to test the stability effect of each differing amino acid residue between the protein of interest and the calculated consensus sequence and to combine all stabilizing mutations into the protein of interest. We used the consensus phytase as protein of interest and tested the effect on the protein stability of 34 amino acid residues, differing to consensus phytase 10 and/or 11 as single mutations.

To construct muteins for expression in A. niger, S. cerevisiae, or H. polymorpha, the corresponding expression plasmid containing the consensus phytase gene was used as template for site-directed mutagenesis (see Example 6-8). Mutations were introduced using the "quick exchangeTM site-directed mutagenesis kit" from Stratagene (La Jolla, CA, USA) following the manufacturer's protocol and using the corresponding primers. All mutations made and their corresponding primers are summarized in Table 4. Plasmids harboring the desired mutation were identified by DNA sequence analysis as known in the art.

Table 4

Primers used for site-directed mutagenesis of consensus phytase

(Exchanged bases are highlighted in bold. The introduction of a restriction site is marked above the sequence. When a

restriction site is written in parenthesis, the mentioned site was destroyed by introduction of the mutation.)

mutation	Primer set
	Kpn I
Q50T	5'-CACTTGTGGGGTACCTACTCTCCATACTTCTC-3'
	5'-GAGAAGTATGGAGAGTAGGTACCCCACAAGTG-3'
Y54F	5'-GGTCAATACTCTCCATTCTTCTCTTTGGAAG-3'
	5'-CTTCCAAAGAGAAGAATGGAGAGTATTGACC-3'
E58A	5'-CATACTTCTCTTTGGCAGACGAATCTGC-3'
	5'-GCAGATTCGTCTGCCAAAGAGAAGTATG-3'
	Aat II
D69K	5'-CTCCAGACGTCCCAAAGGACTGTAGAGTTAC-3'
	5'-GTAACTCTACAGTCCTTTGGGACGTCTGGAG-3'
	Aat II
D70G	5'-CTCCAGACGTCCCAGACGGCTGTAGAGTTAC-3'
	5'-GTAACTCTACAGCCGTCTGGGACGTCTGGAG-3'
K91A	5'-GATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTG-3'
	5'-CAGAGTAAGCCTTAGACGCAGAAGAAGTTGGGTATC-3'
	Sca I
A94K	5'-CTTCTAAGTCTAAGAAGTACTCTGCTTTG-3'
	5'-CAAAGCAGAGTACTTCTTAGACTTAGAAG-3'
A101R	5'-GCTTACTCTGCTTTGATTGAACGGATTCAAAAGAACGCTAC-3'
	5'-GTAGCGTTCTTTTGAATCCGTTCAATCAAAGCAGAGTAAGC-3'
N134Q	5'-CCATTCGGTGAACAGCAAATGGTTAACTC-3'
	5'-GAGTTAACCATTTGCTGTTCACCGAATGG-3'
	Nru I
K153N	5'-GATACAAGGCTCTCGCGAGAAACATTGTTC -3'
	5'-GGAACAATGTTTCTCGCGAGAGCCTTGTATC-3'
	Bss HI
I158V	5'-GATTGTTCCATTCGTGCGCGCTTCTGGTTC-3'
	5'-GAACCAGAAGCGCGCACGAATGGAACAATC-3'

Bcl I

D197N 5'-CTCCAGTTATTAACGTGATCATTCCAGAAGG-3'
5'-CCTTCTGGAATGATCACGTTAATAACTGGAG-3'

Apa I

S187A 5'-GGCTGACCCAGGGGCCCAACCACACCAAGC-3'
5'-GCTTGGTGTGGTTGGGCCCCTGGGTCAGCC-3'

Nco I

T214L 5'-CACTTTGGACCATGGTCTTTGTACTGCTTTCG-3'
5'-CGAAAGCAGTACAAAGACCATGGTCCAAAGTG-3'

Avr II

E222T 5'-GCTTTCGAAGACTCTACCCTAGGTGACGACGTTG-3'
5'-CAACGTCGTCACCTAGGGTAGAGTCTTCGAAAGC-3'

V227A 5'-GGTGACGACGCTGAAGCTAACTTCAC-3'
5'-GTGAAGTTAGCTTCAGCGTCGTCACC-3'

Sac II

L234V 5'-CTAACTTCACCGCGGTGTTCGCTCCAG-3'
5'-CTGGAGCGAACACCGCGGTGAAGTTAG-3'

A238P 5'-GCTTTGTTCGCTCCACCTATTAGAGCTAGATTGG-3'
5'-CCAATCTAGCTCTAATAGGTGGAGCGAACAAAGC-3'

Hpa I

T251N 5'-GCCAGGTGTTAACTTGACTGACGAAG-3'
5'-TTCGTCAGTCAAGTTAACACCTGGC-3'

Aat II

Y259N 5'-GACGAAGACGTCGTTAACTTGATGGAC-3'
5'-GTCCATCAAGTTAACGACGTCTTCGTC-3'

Asp I

E267D 5'-GTCCATTCGACACTGTCGCTAGAACTT C-3'
5'-GAAGTTCTAGCGACAGTGTCGAATGGAC-3'

E277Q 5'-CTGACGCTACTCAGCTGTCTCCATTC-3'
5'-GAATGGAGACAGCTGAGTAGCGTCAG-3'

A283D 5'-GTCTCCATTCTGTGATTTGTTCACTCAC-3'
5'-GTGAGTGAACAAATCACAGAATGGAGAC-3'

Ksp I

H287A 5'-GCTTTGTTCACCGCGGACGAATGGAG-3'
5'-CTCCATTCGTCCGCGGTGAACAAAGC-3'

Bam HI

R291I 5'-CACGACGAATGGATCCAATACGACTAC-3'
5'-GTAGTCGTATTGGATCCATTCGTCGTG-3'

Bsi WI

Q292A 5'-GACGAATGGAGAGCGTACGACTACTTG-3'
5'-CAAGTAGTCGTACGCTCTCCATTCGTC-3'

Hpa I

A320V 5'-GGTGTTGGTTTCGTTAACGAATTGATTGC-3'
5'-GCAATCAATTCGTTAACGAAACCAACACC-3'

(Bgl II)

R329H 5'-GCTAGATTGACTCACTCTCCAGTTCAAG-3'
5'-CTTGAACTGGAGAGTGAGTCAATCTAGC-3'

Eco RV

S364T 5'-CTCACGACAACACTATGATATCTATTTTCTTC-3'
5'-GAAGAAAATAGATATCATAGTGTTGTCGTGAG-3'

Nco I

I366V 5'-CGACAACTCCATGGTTTCTATTTTCTTCGC-3'
5'-GCGAAGAAAATAGAAACCATGGAGTTGTCG-3'

Kpn I

A379K 5'-GTACAACGGTACCAAGCCATTGTCTAC-3'
5'-GTAGACAATGGCTTGGTACCGTTGTAC-3'

S396A 5'-CTGACGGTTACGCTGCTTCTTGGAC-3'
5'-GTCCAAGAAGCAGCGTAACCGTCAG-3'

G404A 5'-CTGTTCCATTCGCTGCTAGAGCTTAC-3'
5'-GTAAGCTCTAGCAGCGAATGGAACAG-3'

Q415E 5'-GATGCAATGTGAAGCTGAAAAGGAACC-3'
5'-GGTTCCTTTTCAGCTTCACATTGCATC-3'

Sal I

A437G 5'-CACGGTTGTGGTGTCGACAAGTTGGG-3'
5'-CCCAACTTGTCGACACCACAACCGTG-3'

Mun I

A463E 5'-GATCTGGTGGCAATTGGGAGGAATGTTTCG-3'
5'-CGAAACATTCCTCCCAATTGCCACCAGATC-3'

and accordingly for other mutations.

The temperature optimum of the purified phytases, expressed in Saccharomyces cerevisiae (Example 7), was determined as outlined in Example 9. Table 5 shows the effect on the stability of consensus phytase for each mutation introduced.

Table 5

Stability effect of the individual amino acid replacements in consensus phytase-1

(+ or - means a positive, respectively, negative effect on the protein stability up to 1°C, ++ and -- means a positive, respectively, negative effect on the protein stability between 1 and 3°C; the number 10 or 11 corresponds to the consensus phytase sequence that suggests the amino acid replacement.)

stabilizing		neutral		destabilizing	
mutation	effect	mutation	effect	mutation	effect
E58A (10)	+	D69A	±	Y54F (10)	-
D69K (11)	+	D70G (10)	±	V73I	-
D197N (10)	+	N134Q (10)	±	A94K (10)	-
T214L (10)	+ +	G186H	±	A101R (11)	-
E222T (11)	+ +	S187A (10)	±	K153N (11)	-
E267D (10)	+	T214V	±	I158V (10)	- -
R291I*	+	T251N (10)	±	G203A	- -
R329H (10)	+	Y259N (10)	±	G205S	-
S364T (10)	+ +	A283D (10)	±	A217V	-
A379K (11)	+	A320V (10)	±	V227A (11)	- -
G404A (10)	+ +	K445T	±	L234V (10)	-
		A463E (10)	±	A238P (10)	- -
				E277Q (10)	-
				H287A (11)	-
				Q292A (10)	-
				I366V (10)	-
				S396A (10)	- -
				Q415E (11)	-
				A437G (10)	- -
				E451R	- -

*: This amino acid replacement was found in another round of mutations.

We combined eight positive mutations (E58A, D197N, E267D, R291I, R329H, S364T, A379K, G404A) in the consensus phytase using the primers and the technique mentioned above in this example. Furthermore, the mutations Q50T and K91A were introduced which mainly influence the catalytical characteristics of the phytase (see patent application EP 97810175.6 and EP 97112688 as well as Example 9). The DNA and amino acid sequence of the resulting phytase gene (consensus phytase-thermo[8]-Q50T-K91A) is shown in Figure 7. In this way, the temperature optimum and the melting point of the consensus phytase was increased by 7°C (Figure 15, 16, 17).

Using the results of Table 5, we further improved the thermostability of consensus phytase 10 by the following back

mutations K94A, V158I, and A396S that revealed a strong negative influence on the stability of consensus phytase. The resulting protein is phytase-10-thermo [3]. Furthermore, we introduced the mutations Q50T and K91A which mainly influence the catalytical characteristics of consensus phytase (see patent application EP 97810175.6 and EP 97112688 as well as Example 9 and Figure 14 and 15). The resulting DNA and amino acid sequence is shown in Figure 8. The optimized phytase showed a 4°C higher temperature optimum and melting point than consensus phytase 10 (Figure 12 and 13). Furthermore, the phytase has also a strongly increased specific activity with phytate as substrate of 250 U/mg at pH 5.5 (Figure 14).

Example 4

Stabilization of the phytase of A. fumigatus ATCC 13073 by replacement of amino acid residues with the corresponding consensus phytase-1 and consensus phytase-10 residues

At six typical positions where the A. fumigatus 13073 is the only or nearly the only phytase in the alignment of Figure 1 that does not contain the corresponding consensus phytase amino acid residue, the non-consensus amino acid residue was replaced by the consensus one. In a first round, the following amino acids were substituted in A. fumigatus 13073 phytase, containing the Q27(24)T substitution and the signal sequence of A. terreus cbs.116.46 phytase (see European Patent Application No. 97810175.6 and Figure 9): F55(28)Y, V100(73)I, F114(87)Y, A243(220)L, S265(242)P, N294(282)D.

The numbers in parentheses confer to the numbering of Figure 1. Number 27 in the mutation Q27(24)T refers to the sequence numbering of Anigmature (phytase of A. niger (ficuum)

NRRL 3135) shown in Fig. 1 of EP 0897010. EP 0897010 is hereby incorporated by reference.

In a second round, four of the seven stabilizing amino acid exchanges (E59A, R329H, S364T, G404A) found in the consensus phytase-10 sequence and, tested as single mutation in consensus phytase-1 (Table 5), were additionally introduced into the A. fumigatus a-mutant. Furthermore, the amino acid replacement S126(154)N, shown to reduce the protease susceptibility of the phytase, was introduced. The number 126 in the mutation S126(154)N refers to the sequence Afummature shown in Fig. 1 of EP0897010.

The mutations were introduced as described in example 3 (see Table 6) and expressed as described in example 6 to 8. The resulting A. fumigatus 13073 phytase variants were called a - mutant (i.e. the A. fumigatus ATCC 13073 phytase with the substitutions Q24T, F28Y, V73I, F87Y, A220L, S242P, N282D) and alpha-mutant or optimized alpha-mutant (i.e. the A. fumigatus alpha-mutant having the additional substitutions E59A-S154N-R329H-S364T-G404A). K68A is an additional preferred mutation.

The temperature optimum (60°C, Figure 20) and the melting point (67.0°C, Figure 19) of the A. fumigatus 13073 phytase alpha-mutant was increased by 5°C in comparison to the values of the wild-type (temperature optimum: 55°C, Tm: 60°C). The five additional amino acid replacements further increased the temperature optimum by 3°C (Figure 20).

Table 6

Mutagenesis primers for stabilization of A. fumigatus phytase ATCC 13073

Mutation	Primer
F55Y	5'-CACGTACTCGCCA**TAC**TTTTCGCTCGAG-3' 5'-CTCGAGCGAAAAGT**ATG**GCGAGTACGTG-3'
E58A	(*Xho* I) 5'-CCATACTTTTCG*CTCGCG*GACGAGCTGTCCGTG-3' 5'-CACGGACAGCTCGTC*CGCGAG*CGAAAAGTAGG-3'
V100I	5'-GTATAAGAAGCT**TATT**ACGGCGATCCAGGCC-3' 5'-GGCCTGGATCGCCGTAA**TAA**GCTTCTTATAC-3'
F114Y	5'-CTTCAAGGGCAAG**TAC**GCCTTTTTGAAGACG-3' 5'-CGTCTTCAAAAAGGC**GTA**CTTGCCCTTGAAG-3'
A243L	5'-CATCCGAGCTCGCC**CTC**GAGAAGCATCTTC-3' 5'-GAAGATGCTTCTC**GAG**GCGAGCTCGGATG-3'
S265P	5'-CTAATGGA TGTGT**CCG**TTTGATACGGTAG-3' 5'-CTACCGTATCAAA**CGG**ACACATGTCCATTAG-3'
N294D	5'-GTGGAAGAAGTA**CGA**CTACCTTCAGTC-3' 5'-GACTGAAGGTAG**TCG**TACTTCTTCCAC-3'
R329H	(*Mlu* I) 5'-GCCCGGTTG*ACGC**A***TTCGCCAGTGCAGG-3' 5'-CCTGCACTGGCGA*ATGCGT*CAACCGGGC-3'
S364T	*Nco* I 5'-CACACGACAACA*CCATGG*TTTCCATCTTC-3' 5'-GAAGATGGAAACCATGGTGTTGTCGTGTG-3'
G404A	(*Bss* HI) 5'-GTGGTGCCTTTCG*CCGCGC*GAGCCTACTTC-3' 5'-GAAGTAGGCTC*GCGCGG*CGAAAGGCACCAC-3'

Example 5

Introduction of the active site amino acid residues of the A. niger NRRL 3135 phytase into the consensus phytase-1

We used the crystal structure of the Aspergillus niger NRRL 3135 phytase to define all active site amino acid residues (see Reference Example and EP 97810175.6). Using the alignment of Figure 1, we replaced the following active site

residues and additionally the not identical adjacent ones of the consensus phytase by that of the A. niger phytase:

S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S

The new protein sequence consensus phytase -7 was backtranslated into a DNA sequence (Figure 10) as described in Example 1. The corresponding gene (fcp7) was generated as described in Example 1 using the following oligonucleotide mixes:

Mix 1.7: CP-1, CP-2, CP-3, CP-4.7, CP-5.7, CP-6, CP-7, CP-8.7, CP-9, CP-10.7

Mix 2.7: CP-9, CP-10.7, CP-11.7, CP-12.7, CP-13.7, CP-14.7, CP-15.7, CP-16, CP-17.7, CP-18.7, CP-19.7, CP-20, CP-21, CP-22.

The DNA sequences of the oligonucleotides are indicated in Figure 3. The newly synthesized oligonucleotides are additionally marked by number 7. After assembling of the oligonucleotides using the same PCR primers as mentioned in Example 1, the gene was cloned into an expression vector as described in Examples 6-8.

The pH-profile determined after expression in H. polymorpha and purification was shifted into the acidic range of the pH-spectrum showing an optimum at pH 4.5-5.0 (see Figure 18). The enzyme had a broad pH-optimum reaching at least 60% of its maximum activity from pH 2.5 to pH 6.0. Up to pH 5.0, the profile resembled the profile of the A. niger NRRL 3135 phytase. However, below pH 5.0 it lacked the typical low at pH 4.0 of the profile of A. niger phytase.

Example 6

Expression of the consensus phytase genes in Hansenula polymorpha

The phytase expression vectors, used to transform H. polymorpha RB11 (Gellissen et al., 1994), was constructed by inserting the Eco RI fragment of pBsk-fcp or variants thereof into the multiple cloning site of the H. polymorpha expression vector pFPMT121, which is based on an ura3 selection marker from S. cerevisiae, a formate dehydrogenase (FMD) promoter element and a methanol oxidase (MO) termimator element from H. polymorpha. The 5′ end of the fcp gene is fused to the FMD promoter, the 3′ end to the MOX terminator (Gellissen et al., 1996; EP 0299 108 B). The resulting expression vector are designated pFPMTfcp, pFPMTfcp10, pFPMTfcp7.

The constructed plasmids were propagated in E. coli. Plasmid DNA was purified using standard state of the art procedures. The expression plasmids were transformed into the H. polymorpha strain RP11 deficient in orotidine-5′-phosphate decarboxylase (ura3) using the procedure for preparation of competent cells and for transformation of yeast as described in Gelissen et al. (1996). Each transformation mixture was plated on YNB (0.14% w/v Difco YNB and 0.5% ammonium sulfate) containing 2% glucose and 1.8% agar and incubated at 37 °C. After 4 to 5 days individual transformant colonies were picked and grown in the liquid medium described above for 2 days at 37 °C. Subsequently, an aliquot of this culture was used to inoculate fresh vials with YNB-medium containing 2% glucose. After seven further passages in selective medium, the expression vector integrates into the yeast genome in multimeric form. Subsequently, mitotically stable transformants were obtained by

two additional cultivation steps in 3 ml non-selective liquid medium (YPD, 2% glucose, 10 g yeast extract, and 20 g peptone). In order to obtain genetically homogeneous recombinant strains an aliquot from the last stabilization culture was plated on a selective plate. Single colonies were isolated for analysis of phytase expression in YNB containing 2% glycerol instead of glucose to derepress the fmd promoter. Purification of the consensus phytases was done as described in Example 7.

Example 7

Expression of the consensus phytase genes in Saccharomyces cerevisiae and purification of the phytases from culture supernatant

The consensus phytase genes were isolated from the corresponding Bluescript-plasmid (pBsk-fcp, pBSK-fcp10, pBsk-fcp7) and ligated into the Eco RI sites of the expression cassette of the Saccharomyces cerevisiae expression vector pYES2 (Invitrogen, San Diego, CA, USA) or subcloned between the shortened GAPFL (glyceraldhyde-3-phosphate dehydrogenase) promoter and the pho5 terminator as described by Janes et al. (1990). The correct orientation of the gene was checked by PCR. Transformation of S. cerevisiae strains. e. g. INVSc1 (Invitrogen, San Diego, CA, USA) was done according to Hinnen et al. (1978). Single colonies harboring the phytase gene under the control of the GAPFL promoter were picked and cultivated in 5 ml selection medium (SD-uracil, Sherman et al., 1986) at 30°C under vigorous shaking (250 rpm) for one day. The preculture was then added to 500 ml YPD medium (Sherman et al., 1986) and grown under the same conditions. Induction of the gal1 promoter was done according to manufacturer's instruction. After four days of incubation cell broth was centrifuged (7000 rpm, GS3 rotor, 15

min, 5°C) to remove the cells and the supernatant was concentrated by way of ultrafiltration in Amicon 8400 cells (PM30 membranes) and ultrafree-15 centrifugal filter devices (Biomax-30K, Millipore, Bedford, MA, USA). The concentrate (10 ml) was desalted on a 40 ml Sephadex G25 Superfine column (Pharmacia Biotech, Freiburg, Germany), with 10 mM sodium acetate, pH 5.0, serving as elution buffer. The desalted sample was brought to 2 M $(NH_4)_2SO_4$ and directly loaded onto a 1 ml Butyl Sepharose 4 Fast Flow hydrophobic interaction chromatography column (Pharmacia Biotech, Feiburg, Germany) which was eluted with a linear gradient from 2 M to 0 M $(NH_4)_2SO_4$ in 10 mM sodium acetate, pH 5.0. Phytase was eluted in the break-through, concentrated and loaded on a 120 ml Sephacryl S-300 gel permeation chromatography column (Pharmacia Biotech, Freiburg, Germany). Consensus phytase and consensus phytase -7 eluted as a homogeneous symmetrical peak and was shown by SDS-PAGE to be approx. 95% pure.

Example 8

Expression of the consensus phytase genes in Aspergillus niger

The Bluescript-plasmids pBsk-fcp, pBSK-fcp10, and pBsk-fcp7 were used as template for the introduction of a Bsp HI-site upstream of the start codon of the genes and an Eco RV-site downstream of the stop codon. The ExpandTM High Fidelity PCR Kit (Boehringer Mannheim, Mannheim, Germany) was used with the following primers:

Primer Asp-1:

Bsp HI

5'-TATA*TCATGA*GCGTGTTCGTCGTGCTACTGTTC-3'

Primer Asp-2 used for cloning of fcp and fcp7:

Eco RV

3'-ACCCGACTTACAAAGCGAATT*CTATAG*ATATAT-5'

Primer Asp-3 used for cloning of fcp10:

Eco RV

3'-ACCCTTCTTACAAAGCGAATT*CTATAG*ATATAT-5'

The reaction was performed as described by the supplier. The PCR-amplified fcp-genes had a new Bsp HI site at the start codon, introduced by primer Asp-1, which resulted in a replacement of the second amino acid residue glycine by serine. Subsequently, the DNA-fragment was digested with Bsp HI and Eco RV and ligated into the Nco I site downstream of the glucoamylase promoter of Aspergillus niger (glaA) and the Eco RV site upstream of the Aspergillus nidulans tryptophan C terminator (trpC) (Mullaney et al., 1985). After this cloning step, the genes were sequenced to detect possible failures introduced by PCR. The resulting expression plasmids which basically corresponds to the pGLAC vector as described in Example 9 of EP 684 313, contained the orotidine-5'-phosphate decarboxylase gene (pyr4) of Neurospora crassa as a selection marker. Transformation of Aspergillus niger and expression of the consensus phytase genes was done as described in EP 684 313. The consensus phytases were purified as described in Example 7.

Example 9

Determination of phytase activity and of temperature optimum

Phytase activity was determined basically as described by Mitchell et al (1997). The activity was measured in an assay mixture containing 0.5% phytic acid (≈5 mM) in 200 mM sodium acetate, pH 5.0. After 15 min of incubation at 37°C, the reaction was stopped by addition of an equal volume of 15% trichloroacetic acid. The liberated phosphate was quantified by mixing 100 µl of the assay mixture with 900 µl H2O and 1 ml of 0.6 M H_2SO_4 , 2% ascorbic acid and 0.5% ammonium molybdate. Standard solutions of potassium phosphate were used as reference. One unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol phosphate per minute at 37°C. The protein concentration was determined using the enzyme extinction coefficient at 280 nm calculated according to Pace et al (1995): consensus phytase, 1.101; consensus phytase 7, 1.068; consensus phytase 10, 1.039.

In case of pH-optimum curves, purified enzymes were diluted in 10 mM sodium acetate, pH 5.0. Incubations were started by mixing aliquots of the diluted protein with an equal volume of 1% phytic acid (≈10 mM) in a series of different buffers: 0.4 M glycine/HCl, pH 2.5; 0.4 M acetate/NaOH, pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5; 0.4 M imidazole/HCl, pH 6.0, 6.5; 0.4 M Tris/HCl pH 7.0, 7.5, 8.0, 8.5, 9.0. Control experiments showed that pH was only slightly affected by the mixing step. Incubations were performed for 15 min at 37°C as described above.

For determinations of the substrate specificities of the phytases, phytic acid in the assay mixture was replaced by 5 mM concentrations of the respective phosphate compounds. The activity tests were performed as described above.

For determination of the temperature optimum, enzyme (100 µl) and substrate solution (100 µl) were pre-incubated for 5 min at the given temperature. The reaction was started by addition of the substrate solution to the enzyme. After 15 min incubation, the reaction was stopped with trichloroacetic acid and the amount of phosphate released was determined.

The pH-optimum of the original consensus phytase was around pH 6.0-6.5 (70 U/mg). By introduction of the Q50T mutation, the pH-optimum shifted to pH 6.0 (130 U/mg). After introduction of K91A, the pH optimum shifted one pH-unit into the acidic pH-range showing a higher specific activity between pH 2.5 and pH 6.0. That was shown for the stabilized mutants and for consensus phytase-10, too (Figure 14 and 15).

Consensus phytase-7, which was constructed to transfer the catalytic characteristics of the A. niger phytase NRRL 3135 into the consensus phytase, had a pH-profile which is shifted into the acidic range of the pH-spectrum showing an optimum between pH 4.5 and 5.0 (see Figure 19). The enzyme had a broad pH-optimum reaching at least 60% of its increased maximum activity from pH 2.5 to pH 6.0. The substrate spectrum, too, resemble more to that of the A. niger NRRL 3135 phytase than to the consensus phytase-1.

The temperature optimum of consensus phytase-1 (71°C) was 16-26°C higher than the temperature optimum of the wild-type phytases (45-55°C, Table 7) which were used to calculate the consensus sequence. The improved consensus phytase-10 showed a further increase of its temperature optimum to 80°C (Figure 11).

The temperature optimum of the consensus phytase-1-thermo[8] was found in the same range (78°C) using the supernatant of an overproducing S. cerevisiae strain. The

highest temperature optimum reached of 82°C was determined for consensus phytase-10-thermo-Q50T-K91A.

Table 7

Temperature optimum and Tm-value of consensus phytase and of the phytases from A. fumigatus, A. niger, E. nidulans, and M. thermophila.

The determination of the temperature optimum was performed as described in Example 9. The Tm-values were determined by differential scanning calorimetry as described in Example 10.

phytase	temperature optimum [°C]	Tm [°C]
Consensus phytase-10-thermo-Q50T-K91A	82	89.3
Consensus phytase-10-thermo-Q50T	82	88.6
Consensus phytase-10	80	85.4
Consensus phytase-1-thermo[8]-Q50T	78	84.7
Consensus phytase-1-thermo[8]-Q50T-K91A	78	85.7
Consensus phytase-1	71	78.1
A. *niger* NRRL3135	55	63.3
A. *fumigatus* 13073	55	62.5
A. *fumigatus* 13073 α-mutant	60	67.0
A. *fumigatus* 13073 α-mutant (optimized)	63	-
A. *terreus* 9A-1	49	57.5
A. *terreus* cbs.116.46	45	58.5
E. nidulans	45	55.7
M. thermophila	55	n. d.
T. thermophilus	45	n. d.

Example 10

Determination of the melting point by differential scanning calorimetry (DSC)

In order to determine the unfolding temperature of the phytases, differential scanning calorimetry was applied as previously published by Brugger et al (1997). Solutions of 50-60 mg/ml homogeneous phytase were used for the tests. A constant heating rate of 10°C/min was applied up to 90-95°C.

The determined melting points reflect the results obtained for the temperature optimums (Table 7). The most stable consensus phytase designed is consensus phytase-10-thermo-Q50T-K91A showing a melting temperature under the choosen condition of 89.3°C. This is 26 to 33.6°C higher than the melting point of the wild-type phytases used.

Example 11

Transfer of basidiomycete phytase active site into consensus phytase-10-thermo-Q50T-K91A

As described previously (Example 3), mutations derived from the basidiomycete phytase active site were introduced into the consensus phytase 10. The following five constructs a) to e) were prepared:

a) This construct is called consensus phytase 12, and it comprises a selected number of active site residues of the basidio consensus sequence, its amino acid sequence (consphy12) is shown in Fig. 21 (the first 26 amino acids forms the signal peptide, amended positions are underlined);

b) a cluster of mutations (Cluster II) was transferred to the consensus 10 sequence, viz.: S80Q, Y86F, S90G, K91A, S92A, K93T, A94R, Y95I;

c) analogously, another cluster of mutations (Cluster III) was transferred, viz.: T129V, E133A, Q143N, M136S, V137S, N138Q, S139A;

d) analogously, a further cluster of mutations (Cluster IV) was transferred, viz.: A168D, E171T, K172N, F173W;

e) and finally, a further cluster of mutations (Cluster V) was transferred, viz.: Q297G, S298D, G300D, Y305T.

These constructs were expressed as described in Examples 6 to 8.

<u>Example 12</u>

<u>Phytase alignment using GAP</u>

The phytases described herein - i.e. the amino acid sequences as well as the corresponding DNA sequences - were aligned against each other. Also some other phytases were correspondingly aligned, viz. the following:

- the consensus phytase described in EP 0897985;
- the phytase derived from Aspergillus niger (ficuum) NRRL 3135 (A. niger NRRL 3135) described in EP 0420358;
- the phytases derived from Aspergillus fumigatus ATCC 13073 (A. fumigatus 13073); Aspergillus fumigatus ATCC 32239 (A. fumigatus 32239); Aspergillus terreus CBS 116.46 (A.terreus cbs); Aspergillus nidulans (E.nidulans); and Talaromyces thermophilus (T. thermophilus) - all described in EP 0897010;
- the phytases derived from Myceliophthora thermophila (M. thermophila); and Aspergillus terreus 9-A1 (A. terreus 9-A1) - both described in EP 0684313;
- the phytase derived from Thermomyces lanuginosus (T.lanuginosus) described in WO 9735017 (PCT/US97/04559);
- the phytases derived from Agrocybe pediades (A. pediades), Paxillus involutus 1 and 2 (P. involutus 1 and 2); and

Trametes pubescens (T. pubescens) - all described i WO 98/28409; and

- the phytase derived from Peniophora lycii (P. lycii) described in WO 98/28408.

For the alignments, the program GAP was used with the settings as described above.

For polypeptide comparisons, the signal peptide were included. However, for alignment to consensus phytase 11, the signal peptides were excluded and only the mature protein part of the other sequences were compared to it.

The results are shown in Table 8 below. The first number in each box or cell is the amino acid similarity, the second number is the amino acid identity.

For DNA sequence comparisons, the signal sequence was included (the same in all phytases).

The results are shown in Table 9 below.

The following embodiments are preferred:

<u>Phytases and corresponding DNA sequences related to consensus phytase 10 (CP10, Fcp 10)</u>

A phytase which comprises an amino acid sequence which is at least 93.80%, preferably at least 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 10 (Fcp10) as shown in Fig. 5.

A phytase which comprises an amino acid sequence which is at least 95.09%, preferably at least 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 10.

A phytase which is encoded by a DNA sequence which is at least 95.88, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (Fcp10) as shown in Fig. 5.

A DNA sequence which encodes a phytase and which (i) is at least 95.88%, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (Fcp10) as shown in Fig. 5. A preferred negative control is DNA encoding consensus phytase. A preferred positive control is DNA encoding any of CP10, CP10-thermo(3)-Q50T, K91A, CP1-thermo(8), CP1-thermo(8)Q50T,K91A.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 93.80%, preferably at least 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 10 (Fcp10) as shown in Fig. 5.

<u>Phytases and corresponding DNA sequences related to consensus phytase 10 thermo(3) Q50T, K91A</u>

A phytase which comprises an amino acid sequence which is at least 93.37%, preferably at least 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8.

A phytase which comprises an amino acid sequence which is at least 94.66%, preferably at least 95.0, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8.

A phytase which is encoded by a DNA sequence which is at least 95.88, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8.

A DNA sequence which encodes a phytase and which (i) is at least 95.88%, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8. A preferred negative control is DNA encoding consensus phytase. A preferred positive control is DNA encoding any of CP10, CP10-thermo(3)-Q50T, K91A, CP1-thermo(8), CP1-thermo(8)Q50T,K91A.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 93.37%, preferably at least 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8.

<u>Phytases and corresponding DNA sequences related to consensus phytase 1-thermo(8)</u>

A phytase which comprises an amino acid sequence which is at least 98.30%, preferably at least 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) (as shown in Fig. 7, backmutations T50Q,A91K to be added).

A phytase which comprises an amino acid sequence which is at least 98.51%, preferably at least 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) (as shown in Fig. 7, backmutations T50Q,A91K to be added).

A phytase which is encoded by a DNA sequence which is at least 98.73, preferably at least 98.5, 99, 99.5% identical to nucleotides 1-1407 of the DNA sequence of consensus phytase 1 thermo(8) (as shown in Fig. 7, backmutations T50Q,A91K to be added).

A DNA sequence which encodes a phytase and which (i) is at least 98.73, preferably at least 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 1-1407 of the DNA sequence of consensus phytase 1 thermo(8) (as shown in Fig. 7, backmutations T50Q,A91K to be added). A preferred negative control is DNA encoding consensus phytase. A preferred positive control is DNA encoding any of CP1-thermo(8), CP1-thermo(8)Q50T,K91A.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 98.30%, preferably at least 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) (as shown in Fig. 7, backmutations T50Q,A91K to be added).

<u>Phytases and corresponding DNA sequences related to consensus phytase 1 thermo(8) Q50T, K91A</u>

A phytase which comprises an amino acid sequence which is at least 97.87%, preferably at least 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7.

A phytase which comprises an amino acid sequence which is at least 98.08%, preferably at least 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7.

A phytase which is encoded by a DNA sequence which is at least 98.37, preferably at least 98.5, 99, 99.5% identical to nucleotides 1-1407 of the DNA sequence of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7.

A DNA sequence which encodes a phytase and which (i) is at least 98.37, preferably at least 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 1-1407 of the DNA sequence of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7. A preferred negative control is DNA encoding consensus phytase. A preferred positive control is DNA encoding any of CP1-thermo(8), CP1-thermo(8)Q50T,K91A.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 97.87%, preferably at least 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7.

<u>Phytases and corresponding DNA sequences related to consensus phytase 11</u>

A phytase which comprises an amino acid sequence which is at least 90.71%, preferably at least 91. 91.5, 92, 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-482 of consensus phytase 11 as shown in Fig. 6.

A phytase which comprises an amino acid sequence which is at least 92.07%, preferably at least 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-482 of consensus phytase 11 as shown in Fig. 6.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 90.71%, preferably at least 91.

91.5, 92, 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-482 of consensus phytase 11 as shown in Fig. 6.

Phytases and corresponding DNA sequences related to A. fumigatus alpha-mutant

A phytase which comprises an amino acid sequence which is at least 97.17%, preferably at least 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of A. fumigatus alpha-mutant (phytase) as shown in Fig. 9.

A phytase which comprises an amino acid sequence which is at least 97.82%, preferably at least 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of A. fumigatus alpha-mutant (phytase) as shown in Fig. 9.

A phytase which is encoded by a DNA sequence which is at least 96.13%, preferably at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 1-1401 of the DNA sequence of A. fumigatus ATCC 13073 alpha-mutant shown in Fig. 9.

A DNA sequence which encodes a phytase comprising an amino acid which is at least 97.17%, preferably at least 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of A. fumigatus ATCC 13073 alpha-mutant shown in Fig. 9.

A DNA sequence which encodes a phytase and which (i) is at least 96.13%, preferably 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 1-1401 of the DNA sequence of A. fumigatus ATCC 13073 alpha-mutant shown in Fig. 9. A preferred negative control is DNA encoding A. fumigatus 13073. A preferred positive control is DNA encoding any of A. fumigatus ATCC 13073 and its optimised alpha-mutant.

<u>Phytases and corresponding DNA sequences related to the optimized A. fumigatus alpha-mutant</u>

A phytase which comprises an amino acid sequence which is at least 96.08%, preferably at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of the phytase of the optimized A. fumigatus alpha-mutant.

A phytase which comprises an amino acid sequence which is at least 96.74%, preferably at least 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of the phytase of the optimized A. fumigatus alpha-mutant.

A phytase which is encoded by a DNA sequence which is at least 95.63%, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 1-1401 of the DNA sequence encoding the phytase of the optimized A. fumigatus alpha-mutant.

A DNA sequence which encodes a phytase comprising an amino acid which is at least 96.08%, preferably at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the phytase of the optimized A. fumigatus alpha-mutant.

A DNA sequence which encodes a phytase and which (i) is at least 95.63%, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 1-1401 of the DNA sequence encoding the phytase of the optimized A. fumigatus alpha-mutant.

A preferred negative control is DNA encoding A. fumigatus 13073. A preferred positive control is DNA encoding any of A. fumigatus ATCC 13073 and its optimised alpha-mutant.

<u>Phytases and corresponding DNA sequences related to consensus phytase 7</u>

A phytase which comprises an amino acid sequence which is at least 94.87%, preferably at least 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 7 as shown in Fig. 10.

A phytase which comprises an amino acid sequence which is at least 95.30%, preferably at least 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 7 as shown in Fig. 10.

A phytase which is encoded by a DNA sequence which is at least 96.38%, preferably 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 7 shown in Fig. 10.

A DNA sequence which encodes a phytase and which (i) is at least 96.38%, preferably at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 12-1412 of the DNA sequence of consensus phytase 7 as shown in Fig. 10.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 94.87%, preferably at least 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 7 as shown in Fig. 10.

Phytases related to basidio consensus

A phytase which comprises an amino acid sequence which is at least 76.23%, preferably at least 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the combined sequence of (i) amino acids 1-441 of basidio consensus shown in

Fig. 3, and (ii) amino acids 1-26 shown in Fig. 5 (the sequence of (ii) to be added at the N-terminal of the sequence of (i)).

A phytase which comprises an amino acid sequence which is at least 79.50%, preferably at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-441 of basidio consensus as shown in Fig. 3.

Phytases related to consensus phytase 12

A phytase which comprises an amino acid sequence which is at least 70, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 12 as shown in Fig. 21.

A phytase which comprises an amino acid sequence which is at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 12 as shown in Fig. 21.

Table 8

Comparison of phytase amino acid sequences

Phytase	CP10	CP10-thermo[3]Q50T-K91A	CP1-thermo[8]	CP1-thermo[8]-Q50T-K91A	CP11	CP7	Basidio	A. fumigatus a-mutant	A. fumi-gatus a-mutant (opt.)
Consensus phytase	95.08/93.79	94.65/93.36	98.50/98.29	98.07/97.86	92.06/90.70	95.29/94.86	69.42/62.16	85.59/82.58	84.73/81.72
A. niger NRRL3135	79.48/76.46	79.05/76.03	80.35/77.75	79.91/77.32	79.27/76.31	84.02/81.64	67.19/59.32	74.07/70.11	74.95/70/99
A. terreus 9-A1	76.04/72.11	75.82/71.90	76.47/72.33	76.25/72.11	76.51/73.02	75.76/71.18	65.39/58.02	69.67/64.84	69.45/64.84
A. terreus cbs	79.04/75.11	78.82/74.89	79.48/75.76	79.26/75.55	77.19/73.27	79.17/75.00	66.92/59.65	72.59/67.76	72.37/67.76
E. nidulans	78.70/74.35	78.26/73.91	79.78/75.87	79.35/75.44	80.56/76.62	76.96/73.04	67.20/58.13	72.39/67.83	72.11/67.54
A. fumigatus 13073	82.93/80.31	82.50/79.87	82.31/79.04	81.88/78.60	81.36/78.64	80.13/76.20	63.54/57.91	97.82/97.16	96.73/96.07
A. fumigatus 32239	81.30/77.39	80.87/76.96	81.09/77.61	80.65/77.17	79.95/76.08	79.13/75.22	63.61/54.97	90.22/86.52	89.57/85.87
T. thermophilus	77.83/73.84	77.38/73.39	78.67/74.89	78.22/74.44	78.47/74.76	76.51/73.15	61.54/54.36	72.01/66.82	72.69/67.49
M . thermophila	69.16/62.81	69.48/63.33	69.27/62.84	69.59/63.36	69.65/63.06	68.82/62.13	65.56/57.91	66.21/58.45	66.44/58.68
T. lanuginosus	73.52/66.70	73.06/66.44	71.92/64.61	71.46/64.16	74.21/68.86	69.50/62.62	67.20/57.41	68.91/61.02	69.61/61.72
P. lycii	64.92/59.10	64.91/59.37	64.46/58.09	64.46/58.36	65.03/59.84	63.13/56.50	77.75/73.07	64.08/57.11	62.47/55.91
A. pediades	64.51/51.81	64.86/51.94	62.98/51.41	63.33/51.54	64.50/52.30	63.05/51.15	78.92/74.71	61.64/52.38	62.13/53.07
P. involutus 1	66.67/58.07	66.67/58.33	64.84/56.51	64.84/56.77	63.30/54.52	65.33/56.53	79.49/76.22	59.59/51.81	59.95/52.20
P. involutus 2	65.54/55.70	65.30/55.53	66.85/56.87	66.58/56.68	66.30/56.35	64.27/54.13	78.09/74.59	61.26/52.62	61.04/52.47

T. pubescens	65.46/57.22	65.72/57.47	62.89/55.67	63.14/55.93	65.03/57.65	63.28/56.51	78.34/75.12	64.08/57.11	62.30/55.24
CP10	-	99.57/99.57	96.57/95.50	96.15/95.08	95.02/94.56	91.01/89.29	70.22/62.28	85.13/82.76	85.99/83.62
CP10t[3]Q50TK91A	99.57/99.57	-	96.15/95.08	96.57/95.50	94.56/94.10	90.58/88.87	70.47/62.28	85.13/82.76	85.99/83.62
CP1thermo[8]	96.57/95.50	96.15/95.08	-	99.57/99.57	93.42/92.29	94.43/93.79	68.40/60.74	84.52/81.94	85.38/82.80
CP1t[8]Q50TK91A	96.15/95.08	96.57/95.50	99.57/99.57	-	92.97/91.84	94.00/93.36	68.64/60.74	84.52/81.94	85.38/82.80
CP11	95.02/94.56	94.56/94.10	93.42/92.29	92.97/91.84	-	88.44/86.62	68.27/59.73	82.23/79.73	83.37/80.87
CP7	91.01/89.29	90.58/88.87	94.43/93.79	94.00/93.36	88.44/86.62	-	69.80/62.69	81.94/78.71	81.72/78.50
Basidio	70.22/62.28	70.47/62.28	68.40/60.74	68.64/60.74	68.27/59.73	69.80/62.69	-	65.97/60.52	66.41/60.68
A.fumigatus a-mut.	85.13/82.76	85.13/82.76	84.52/81.94	84.52/81.94	82.23/79.73	81.94/78.71	65.97/60.52	-	98.93/98.93
A. fum a-mut -opt.	85.99/83.62	85.99/83.62	85.38/82.80	85.38/82.80	83.37/80.87	81.72/78.50	66.41/60.68	98.93/98.93	-

Table 9

Comparison of phytase encoding DNA sequences

Phytase	CP10	CP10-thermo[3]Q50T-K91A	CP1-thermo[8]	CP1-thermo[8]-Q50T-K91A	CP7	Basidio	A. fumigatus a-mutant	A. fumi-gatus a-mutant (opt.)
Consensus phytase	95.87	95.87	98.72	98.36	96.37	65.46	66.88	66.88
A. niger NRRL3135	65.10	64.82	66.10	65.74	67.52	50.68	65.88	66.17
A. terreus 9-A1	61.74	61.53	62.17	62.03	60.53	49.40	66.24	66.31
A. terreus cbs	62.52	62.30	63.02	62.88	61.45	49.74	68.17	68.24
E. nidulans	65.08	64.94	65.30	65.01	64.22	49.92	64.90	65.44
A. fumigatus 13073	65.66	65.38	64.19	64.08	63.65	48.27	96.12	95.62
T. thermophilus	62.52	62.50	62.53	62.66	62.00	52.19	61.77	61.92
M . thermophila	55.51	55.15	55.36	55.22	53.91	48.44	58.17	58.24
T. lanuginosus	57.56	57.20	56.76	56.47	62.00	44.66	59.71	60.07
P. lycii	45.76	46.51	45.14	55.21	55.46	58.50	48.91	49.44
A. pediades	49.89	49.89	49.89	50.11	45.54	61.66	47.49	47.56
P. involutus 1	48.32	49.03	47.81	47.96	49.59	59.80	49.96	50.19
P. involutus 2	48.24	49.00	48.08	48.63	47.94	60.16	47.56	47.63
T. pubescens	47.00	47.17	46.46	47.62	46.83	60.37	49.89	49.96
CP10	-	99.43	96.40	96.05	93.73	66.40	67.81	68.24

CP10t[3]Q50TK91A	99.43	-	96.37	96.58	93.45	66.29	67.81	68.24
Cp1thermo[8]	96.40	96.37	-	99.65	95.30	65.40	66.74	67.17
CP1t[8]Q50TK91A	96.05	96.58	99.65	-	94.94	65.47	66.74	67.17
CP7	93.73	93.45	95.30	94.94	-	64.56	65.88	65.88
Basidio	66.40	66.29	65.40	65.47	64.56	-	50.41	50.49
A.fumigatus a-mut.	67.81	67.81	66.74	66.74	65.88	50.41	-	99.50
A. fum a-mut -opt.	68.24	68.24	67.17	67.17	65.88	50.49	99.50	-

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SEQUENCE LISTING

to follow!

CLAIMS

1. A phytase which comprises an amino acid sequence which is at least 93.80% identical to the sequence of amino acids 1-467 of consensus phytase 10 (Fcp10) as shown in Fig. 5.

2. A phytase which is encoded by a DNA sequence which is at least 95.88% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (Fcp10) as shown in Fig. 5.

3. A phytase which comprises an amino acid sequence selected from amongst the amino acid sequence of Fig. 5, and the amino acid sequence designated Fcp10 shown in Fig. 4 (consensus phytase 10).

4. A phytase which comprises an amino acid sequence selected from amongst the amino acid sequences of (i) consensus phytase-10-thermo[3] (also designated consensus phytase-10-thermo); (ii) the variant Q50T, (iii) K91A, or (iv) (Q50T+K91A) thereof - variant (iv) is shown in Fig. 8; and (v) amino acids 27-467 of any of the sequences (i) to (iv).

5. A phytase which comprises an amino acid sequence selected from amongst the amino acid sequences of (i) consensus phytase-1-thermo[8] (also designated consensus phytase-1-thermo); (ii) the variant Q50T, (iii) K91A, or (iv) (Q50T+K91A) thereof - variant (iv) is shown in Fig. 7; and (v) amino acids 27-467 of any of the sequences of (i) to (iv).

6. A phytase which comprises the amino acid sequence of consensus phytase-11 shown in Fig. 6.

7. A DNA sequence which encodes the phytase of any one of claims 1-6.

8. A DNA sequence which encodes a phytase, and which is (i) at least 95.88% identical, or (ii) hybridizes under high stringency conditions, to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (Fcp10) as shown in Fig. 5.

9. A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 93.80% identical to the sequence of amino acids 1-467 of consensus phytase 10 (Fcp10) as shown in Fig. 5.

10. A DNA sequence which encodes a phytase, and which comprises

(i) nucleotides 1-1426, 12-1412, 90-1426 or 90-1412 of the DNA sequence encoding consensus phytase 10 (Fcp 10) shown in Fig. 5;

(ii) nucleotides 1-1404, 1-1401, 79-1404, or 79-1401 of the DNA sequence encoding consensus phytase-10-thermo[3]-Q50T, K91A shown in Fig. 8;

(iii) variants of the nucleotides of (ii) which encodes variants (i)-(iii) of claim 4;

(iv) nucleotides 1-1410, 1-1407, 79-1410 or 79-1407 of the DNA sequence encoding consensus phytase-1-thermo[8]-Q50T, K91A as shown in Fig. 7;

(v) variants of the nucleotides of (iv) which encodes variants (i)-(iii) of claim 5; or

11. A vector comprising the DNA sequence according to any one of claims 3-5.

12. A host cell comprising the DNA sequence according to any one of claims 3-5 or the vector according to claim 6.

13. A process for producing a phytase, the process comprising culturing the host cell according to claim 7 under conditions permitting the production of the phytase, and recovering the phytase from the culture broth.

14. A food, feed or pharmaceutical composition comprising the phytase of any one of claims 1-2.

ABSTRACT

This invention relates to improved phytases, preferably phytases of an increased thermostability, and a process of producing them based on a comparison with consensus phytases. In particular, stabilizing amino acid mutations are introduced into a homologous protein, or the active site of a phytase is replaced in part or in toto. The corresponding DNA sequences and methods of preparing it is also disclosed, as are methods of producing the improved phytases, and the use thereof. Specific variants of Aspergillus fumigatus phytase and consensus phytases are disclosed.

Figure 1

	1				50
A. terreus 9A-1	KhsDCNSVDh	GYQCFPELSH	kWGlYAPYFS	LQDESPFPlD	VPEDChITFV
A. terreus cbs	NhsDCTSVDr	GYQCFPELSH	kWGlYAPYFS	LQDESPFPlD	VPDDChITFV
A. niger var. *awamori*	NqsTCDTVDQ	GYQCFSETSH	LWGQYAPFFS	LANESAISPD	VPAGCrVTFA
A. niger T213	NqsSCDTVDQ	GYQCFSETSH	LWGQYAPFFS	LANESVISPD	VPAGCrVTFA
A. niger NRRL3135	NqsSCDTVDQ	GYQCFSETSH	LWGQYAPFFS	LANESVISPE	VPAGCrVTFA
A. fumigatus 13073	GSkSCDTVDl	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
A. fumigatus 32722	GSkSCDTVDl	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
A. fumigatus 58128	GSkSCDTVDl	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
A. fumigatus 26906	GSkSCDTVDl	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
A. fumigatus 32239	GSkACDTVEl	GYQCsPGTSH	LWGQYSPFFS	LEDElSVSSD	LPKDCrVTFV
E. nidulans	QNHSCNTADG	GYQCFPNVSH	VWGQYSPYFS	IEQESAISeD	VPHGCeVTFV
T. thermophilus	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPQNCkITFV
M. thermophila	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS	VpSElDaS..	IPDDCeVTFA
Consensus	NSHSCDTVDG	GYQCFPEISH	LWGQYSPYFS	LEDESAISPD	VPDDC-VTFV
Consensus phytase	**NSHSCDTVDG**	**GYQCFPEISH**	**LWGQYSPYFS**	**LEDESAISPD**	**VPDDCRVTFV**

	51				100
A. terreus 9A-1	QVLARHGARs	PThSKtKAYA	AtIAAIQKSA	TaFpGKYAFL	QSYNYSLDSE
A. terreus cbs	QVLARHGARs	PTDSKtKAYA	AtIAAIQKNA	TaLpGKYAFL	KSYNYSMGSE
A. niger var. *awamori*	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV	TtFDGKYAFL	KTYNYSLGAD
A. niger T213	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV	TtFDGKYAFL	KTYNYSLGAD
A. niger NRRL3135	QVLSRHGARY	PTDSKgKkYS	ALIEEIQQNA	TtFDGKYAFL	KTYNYSLGAD
A. fumigatus 13073	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 32722	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 58128	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 26906	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 32239	QVLSRHGARY	PTASKsKkYK	kLVTAIQKNA	TeFKGKFAFL	ETYNYTLGAD
E. nidulans	QVLSRHGARY	PTESKsKAYS	GLIEAIQKNA	TsFwGQYAFL	ESYNYTLGAD
T. thermophilus	QLLSRHGARY	PTSSKtElYS	QLISrIQKTA	TaYKGyYAFL	KDYrYqLGAN
M. thermophila	QVLSRHGARa	PTlKRaaSYv	DLIDrIHhGA	IsYgPgYEFL	RTYDYTLGAD
Consensus	QVLSRHGARY	PTSSK-KAYS	ALIEAIQKNA	T-FKGKYAFL	KTYNYTLGAD
Consensus phytase	**QVLSRHGARY**	**PTSSKSKAYS**	**ALIEAIQKNA**	**TAFKGKYAFL**	**KTYNYTLGAD**

	101				150
A. terreus 9A-1	ELTPFGrNQL	rDlGaQFYeR	YNALTRhInP	FVRATDASRV	hESAEKFVEG
A. terreus cbs	NLTPFGrNQL	qDlGaQFYRR	YDTLTRhInP	FVRAADSSRV	hESAEKFVEG
A. niger var. *awamori*	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIIP	FIRSSGSSRV	IASGEKFIEG
A. niger T213	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIIP	FIRSSGSSRV	IASGEKFIEG
A. niger NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIVP	FIRSSGSSRV	IASGKKFIEG
A. fumigatus 13073	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. fumigatus 32722	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. fumigatus 58128	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. fumigatus 26906	DLTAFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. fumigatus 32239	DLTPFGEQQM	VNSGIKFYQK	YKALAgSVVP	FIRSSGSDRV	IASGEKFIEG
E. nidulans	DLTiFGENQM	VDSGaKFYRR	YKNLARKnTP	FIRASGSDRV	VASAEKFING
T. thermophilus	DLTPFGENQM	IQlGIKFYnH	YKSLARNaVP	FVRCSGSDRV	IASGrlFIEG
M. thermophila	ELTRtGQQQM	VNSGIKFYRR	YRALARKsIP	FVRTAGqDRV	VhSAENFTQG
Consensus	DLTPFGENQM	VNSGIKFYRR	YKALARK-VP	FVRASGSDRV	IASAEKFIEG
Consensus phytase	**DLTPFGENQM**	**VNSGIKFYRR**	**YKALARKIVP**	**FIRASGSDRV**	**IASAEKFIEG**

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                         151                                                   200
A. terreus 9A-1          FQTARqDDHh ANpHQPSPrV DVaIPEGSAY NNTLEHSlCT AFES...STV
A. terreus cbs           FQNARqGDPh ANpHQPSPrV DVVIPEGTAY NNTLEHSICT AFEA...STV
A. niger var. awamori    FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL
A. niger T213            FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL
A. niger NRRL3135        FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL
A. fumigatus 13073       FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT kFEA...SQL
A. fumigatus 32722       FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT kFEA...SQL
A. fumigatus 58128       FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT kFEA...SQL
A. fumigatus 26906       FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT kFEA...SQL
A. fumigatus 32239       FQqANVADPG A.TNRAAPVI SVIIPESETY NNTLDHSVCT NFEA...SEL
E. nidulans              FRKAQLhDHG S..gQATPVV NVIIPEiDGF NNTLDHSTCV SFEN...DEr
T. thermophilus          FQSAKVlDPh SDkHDAPPTI NVIIeEGPSY NNTLDtGSCP VFED...SSg
M. thermophila           FHSAlLADRG STvRPTlPyd mVVIPETAGa NNTLHNDlCT AFEEgpySTI

Consensus                FQSAKLADPG S-PHQASPVI NVIIPEGSGY NNTLDHGTCT AFED---SEL
Consensus phytase        FQSAKLADPG SQPHQASPVI DVIIPEGSGY NNTLDHGTCT AFED...SEL
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                         201                                                   250
A. terreus 9A-1          GDDAvANFTA VFAPAIaQRL EADLPGVqLS TDDVVnLMAM CPFETVSlTD
A. terreus cbs           GDAAADNFTA VFAPAIakRL EADLPGVqLS ADDVVnLMAM CPFETVSlTD
A. niger var. awamori    ADTVEANFTA TFAPSIRQRL ENDLSGVTLT DTEVTyLMDM CSFDTIStST
A. niger T213            ADTVEANFTA TFAPSIRQRL ENDLSGVTLT DTEVTyLMDM CSFDTIStST
A. niger NRRL3135        ADTVEANFTA TFVPSIRQRL ENDLSGVTLT DTEVTyLMDM CSFDTIStST
A. fumigatus 13073       GDEVAANFTA lFAPDIRARa EkHLPGVTLT DEDVVsLMDM CSFDTVARTS
A. fumigatus 32722       GDEVAANFTA lFAPDIRARa EkHLPGVTLT DEDVVsLMDM CSFDTVARTS
A. fumigatus 58128       GDEVAANFTA lFAPDIRARa EkHLPGVTLT DEDVVsLMDM CSFDTVARTS
A. fumigatus 26906       GDEVAANFTA lFAPDIRARa KkHLPGVTLT DEDVVsLMDM CSFDTVARTS
A. fumigatus 32239       GDEVEANFTA lFAPAIRARI EkHLPGVqLT DDDVVsLMDM CSFDTVARTA
E. nidulans              ADEiEANFTA IMGPPIRkRL ENDLPGIKLT NENVIyLMDM CSFDTMARTA
T. thermophilus          GHDAQEKFAk qFAPAIlEKI KDHLPGVDLA vSDVpyLMDL CPFETLARNh
M. thermophila           GDDAQDTYlS TFAGPItARV NANLPGANLT DADTVaLMDL CPFETVAsSS

Consensus                GDDAEANFTA TFAPAIRARL EADLPGVTLT DEDVV-LMDM CPFETVARTS
Consensus phytase        GDDVEANFTA LFAPAIRARL EADLPGVTLT DEDVVYLMDM CPFETVARTS
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                         251                                                   300
A. terreus 9A-1          ......... ...DAhTLSPFC DLFTAtEWtq YNYLlSLDKY YGYGGGNPLG
A. terreus cbs           ......... ...DAhTLSPFC DLFTAaEWtq YNYLlSLDKY YGYGGGNPLG
A. niger var. awamori    ......... ...vDTKLSPFC DLFTHdEWih YDYLQSLkKY YGHGAGNPLG
A. niger T213            ......... ...vDTKLSPFC DLFTHdEWih YDYLRSLkKY YGHGAGNPLG
A. niger NRRL3135        ......... ...vDTKLSPFC DLFTHdEWin YDYLQSLkKY YGHGAGNPLG
A. fumigatus 13073       ......... ...DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
A. fumigatus 32722       ......... ...DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
A. fumigatus 58128       ......... ...DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
A. fumigatus 26906       ......... ...DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
A. fumigatus 32239       ......... ...DASELSPFC AIFTHnEWkk YDYLQSLGKY YGYGAGNPLG
E. nidulans              ......... ...HGTELSPFC AIFTEkEWlq YDYLQSLSKY YGYGAGSPLG
T. thermophilus          ......... ...TDT.LSPFC ALsTQeEWqa YDYYQSLGKY YGnGGGNPLG
M. thermophila           sdpatadagg gNGrpLSPFC rLFSEsEWra YDYLQSVGKW YGYGPGNPLG

Consensus                ---------- -DATELSPFC ALFTE-EW-- YDYLQSLGKY YGYGAGNPLG
Consensus phytase        .......... .DATELSPFC ALFTHDEWRQ YDYLQSLGKY YGYGAGNPLG
```

	301				350
A. terreus 9A-1	PVQGVGWaNE	LMARLTRAPV	HDHTCVNNTL	DASPATFPLN	ATLYADFSHD
A. terreus cbs	PVQGVGWaNE	LIARLTRSPV	HDHTCVNNTL	DANPATFPLN	ATLYADFSHD
A. niger var. *awamori*	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSNPATFPLN	STLYADFSHD
A. niger T213	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSNPATFPLN	STLYADFSHD
A. niger NRRL3135	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSSPATFPLN	STLYADFSHD
A. fumigatus 13073	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 32722	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 58128	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 26906	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 32239	PAQGIGFtNE	LIARLTNSPV	QDHTSTNsTL	DSDPATFPLN	ATIYVDFSHD
E. nidulans	PAQGIGFtNE	LIARLTQSPV	QDNTSTNHTL	DSNPATFPLD	rKLYADFSHD
T. thermophilus	PAQGVGFvNE	LIARMTHSPV	QDYTTVNHTL	DSNPATFPLN	ATLYADFSHD
M. thermophila	PTQGVGFvNE	LLARLAgvPV	RDgTSTNRTL	DGDPrTFPLG	rPLYADFSHD
Consensus	PAQGVGF-NE	LIARLTHSPV	QDHTSTNHTL	DSNPATFPLN	ATLYADFSHD
Consensus phytase	**PAQGVGFANE**	**LIARLTRSPV**	**QDHTSTNHTL**	**DSNPATFPLN**	**ATLYADFSHD**

	351				400
A. terreus 9A-1	SNLVSIFWAL	GLYNGTAPLS	qTSVESVSQT	DGYAAAWTVP	FAARAYVEMM
A. terreus cbs	SNLVSIFWAL	GLYNGTkPLS	qTTVEDITrT	DGYAAAWTVP	FAARAYIEMM
A. niger var. *awamori*	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRlYVEMM
A. niger T213	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRlYVEMM
A. niger NRRL3135	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRlYVEMM
A. fumigatus 13073	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFEtM
A. fumigatus 32722	NSMVSIFFAL	GLYNGTGPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFEtM
A. fumigatus 58128	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFEtM
A. fumigatus 26906	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFEtM
A. fumigatus 32239	NGMIPIFFAM	GLYNGTEPLS	qTSeESTKES	NGYSASWAVP	FGARAYFEtM
E. nidulans	NSMISIFFAM	GLYNGTQPLS	mDSVESIQEm	DGYAASWTVP	FGARAYFELM
T. thermophilus	NTMTSIFaAL	GLYNGTAkLS	TTEIKSIEET	DGYSAAWTVP	FGGRAYIEMM
M. thermophila	NDMMGVLgAL	GaYDGVPPLD	KTArrDpEEl	GGYAASWAVP	FAARiYVEKM
Consensus	NSMISIFFAL	GLYNGTAPLS	TTSVESIEET	DGYAASWTVP	FGARAYVEMM
Consensus phytase	**NSMISIFFAL**	**GLYNGTAPLS**	**TTSVESIEET**	**DGYSASWTVP**	**FGARAYVEMM**

	401				450
A. terreus 9A-1	QC........	.....RAEKE	PLVRVLVNDR	VMPLHGCPTD	KLGRCKrDAF
A. terreus cbs	QC........	.....RAEKQ	PLVRVLVNDR	VMPLHGCAVD	NLGRCKrDDF
A. niger var. *awamori*	QC........	.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. niger T213	QC........	.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. niger NRRL3135	QC........	.....QAEQE	PLVRVLVNDR	VVPLHGCPVD	aLGRCTrDSF
A. fumigatus 13073	QC........	.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDF
A. fumigatus 32722	QC........	.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDF
A. fumigatus 58128	QC........	.....KSEKE	SLVRALINDR	VVPLHGCDVD	KLGRCKLNDF
A. fumigatus 26906	QC........	.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDF
A. fumigatus 32239	QC........	.....KSEKE	PLVRALINDR	VVPLHGCAVD	KLGRCKLKDF
E. nidulans	QC........	.....E.KKE	PLVRVLVNDR	VVPLHGCAVD	KFGRCTLDDW
T. thermophilus	QC........	.....DDSDE	PVVRVLVNDR	VVPLHGCEVD	SLGRCKrDDF
M. thermophila	RCsggggggg	ggegrQEKDE	eMVRVLVNDR	VMTLkGCGAD	ErGMCTLErF
Consensus	QC--------	-----QAEKE	PLVRVLVNDR	VVPLHGCAVD	KLGRCKLDDF
Consensus phytase	**QC........**	**.....QAEKE**	**PLVRVLVNDR**	**VVPLHGCAVD**	**KLGRCKRDDF**

	451	471
A. terreus 9A-1	VAGLSFAQAG	GNWADCF--- -
A. terreus cbs	VEGLSFARAG	NWAECF---
A. niger var. *awamori*	VrGLSFARSG	GDWAECsA-- -
A. niger T213	VrGLSFARSG	GDWAECFA-- -
A. niger NRRL3135	VrGLSFARSG	DWAECFA--
A. fumigatus 13073	VKGLSWARSG	GNWGECFS-- -
A. fumigatus 32722	VKGLSWARSG	GNWGECFS-- -
A. fumigatus 58128	VKGLSWARSG	GNWGECFS-- -
A. fumigatus 26906	VKGLSWARSG	GNWGECFS-- -
A. fumigatus 32239	VKGLSWARSG	NSEQSFS--
E. nidulans	VEGLNFARSG	GNWkTCFTl- -
T. thermophilus	VrGLSFARqG	GNWEGCYAas e
M. thermophila	IESMAFARGN	GKWDlCFA-- -
Consensus	VEGLSFARSG	GNWAECFA-- -
Consensus phytase	**VEGLSFARSG**	**GNWAECFA.. .**

Figure 2

```
      CP-1
       Eco RI  M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T
      TATATGAATTCATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCA
    1 ---------+---------+---------+---------+---------+---------+ 60
      ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT

       S  G  T  A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G
      CATCCGGTACCGCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG
   61 ---------+---------+---------+---------+---------+---------+ 120
      GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC
           CP-2
                CP-3
       Y  Q  C  F  P  E  I  S  H  L  W  G  Q  Y  S  P  Y  F  S  L
      GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT
  121 ---------+---------+---------+---------+---------+---------+ 180
      CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTATGAAGAGAA

       E  D  E  S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q
      TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTC
  181 ---------+---------+---------+---------+---------+---------+ 240
      ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG
                     CP-4
                          CP-5
       V  L  S  R  H  G  A  R  Y  P  T  S  S  K  S  K  A  Y  S  A
      AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTTCTTCTAAGTCTAAGGCTTACTCTG
  241 ---------+---------+---------+---------+---------+---------+ 300
      TTCAAAACAGATCTGTGCCACGATCTATGGGTTGAAGAAGATTCAGATTCCGAATGAGAC

       L  I  E  A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K
      CTTTGATTGAAGCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
  301 ---------+---------+---------+---------+---------+---------+ 360
      GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT
                               CP-6
                                    CP-7
       T  Y  N  Y  T  L  G  A  D  D  L  T  P  F  G  E  N  Q  M  V
      AGACTTACAACTACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGG
  361 ---------+---------+---------+---------+---------+---------+ 420
      TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC

       N  S  G  I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F
      TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT
  421 ---------+---------+---------+---------+---------+---------+ 480
      AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA
                                                   CP-8
                                                        CP-9
       I  R  A  S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F
      TCATTAGAGCTTCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT
  481 ---------+---------+---------+---------+---------+---------+ 540
      AGTAATCTCGAAGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

       Q  S  A  K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  D
      TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG
  541 ---------+---------+---------+---------+---------+---------+ 600
      AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAAC
```

```
                                                         CP-10
                                                             CP-11
     V  I  I  P  E  G  S  G  Y  N  N  T  L  D  H  G  T  C  T  A
    ACGTTATTATTCCAGAAGGaTCcGGTTACAACAACACTTTGGACCACGGTACTTGTACTG
601 ---------+---------+---------+---------+---------+---------+ 660
    TGCAATAATAAGGTCTTCCtAGgCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGAC

      F  E  D  S  E  L  G  D  D  V  E  A  N  F  T  A  L  F  A  P
    CTTTCGAAGACTCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTCGCTC
661 ---------+---------+---------+---------+---------+---------+ 720
    GAAAGCTTCTGAGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAG
                                                            CP-12

     A  I  R  A  R  L  E  A  D  L  P  G  V  T  L  T  D  E  D  V
    CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACG
721 ---------+---------+---------+---------+---------+---------+ 780
    GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGCTTCTGC

    CP-13
     V  Y  L  M  D  M  C  P  F  E  T  V  A  R  T  S  D  A  T  E
    TTGTTTACTTGATGGACATGTGTCCATTCGAAACTGTTGCTAGAACTTCTGACGCTACTG
781 ---------+---------+---------+---------+---------+---------+ 840
    AACAAATGAACTACCTGTACACAGGTAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

     L  S  P  F  C  A  L  F  T  H  D  E  W  R  Q  Y  D  Y  L  Q
    AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGACAATACGACTACTTGC
841 ---------+---------+---------+---------+---------+---------+ 900
    TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTGTTATGCTGATGAACG
         CP-14
             CP-15
     S  L  G  K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V
    AATCTTTGGGTAAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTG
901 ---------+---------+---------+---------+---------+---------+ 960
    TTAGAAACCCATTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCAC

     G  F  A  N  E  L  I  A  R  L  T  R  S  P  V  Q  D  H  T  S
    TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT
961 ---------+---------+---------+---------+---------+---------+ 1020
    AACCAAAGCGATTGCTTAACTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA
                 CP-16
                     CP-17
     T  N  H  T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A
    CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG
1021 ---------+---------+---------+---------+---------+---------+ 1080
    GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

     D  F  S  H  D  N  S  M  I  S  I  F  F  A  L  G  L  Y  N  G
    CTGACTTCTCTCACGACAACTCTATGATTTCTATTTTCTTCGCTTTGGGTTTGTACAACG
1081 ---------+---------+---------+---------+---------+---------+ 1140
    GACTGAAGAGAGTGCTGTTGAGATACTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC
                         CP-18
                             CP-19
     T  A  P  L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A
    GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTG
1141 ---------+---------+---------+---------+---------+---------+ 1200
    CATGACGAGGTAACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGAC
```

```
      S  W  T  V  P  F  G  A  R  A  Y  V  E  M  M  Q  C  Q  A  E
     CTTCTTGGACTGTTCCATTCGGTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
1201 ---------+---------+---------+---------+---------+---------+ 1260
     GAAGAACCTGACAAGGTAAGCCACGATCTCGAATGCAACTTTACTACGTTACAGTTCGAC
                                                 CP-20
                                                      CP-21
      K  E  P  L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A
     AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC

                V  D  K  L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A
R
     CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA
1321 ---------+---------+---------+---------+---------+---------+ 1380
     GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
                                                           CP-22
      S  G  G  N  W  A  E  C  F  A  *  Eco RI
     GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA
1381 ---------+---------+---------+---------+------ 1426
     CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT
```

Figure 3

	1				50
P. involutus (phyA1)	SvP.KnTAPt	FPIPeseQrn	WSPYSPYFPL	AeYkAPPAGC	QInQVNIIQR
P. involutus (phyA2)	SvP.RniAPK	FSIPeseQrn	WSPYSPYFPL	AeYkAPPAGC	EInQVNIIQR
T. pubescens	hiPlRdTSAc	LdVTrDvQqs	WSmYSPYFPa	AtYvAPPASC	QInQVHIIQR
A. pediades	GgvvQaTfvQ	pfFPpQiQds	WAAYTPYYPV	qaYtPPPkDC	KItQVNIIQR
P. lycii	StQfsfvAAQ	LPIPaQntsn	WGPYdPFFPV	EpYaAPPEGC	tVtQVNLIQR
Basidio	**S-P-R-TAAQ**	**LPIP-Q-Q--**	**WSPYSPYFPV**	**A-Y-APPAGC**	**QI-QVNIIQR**

	51				100
P. involutus (phyA1)	HGARFPTSGA	TTRIKAGLTK	LQGvqnfTDA	KFNFIkSfkY	dLGnsDLVPF
P. involutus (phyA2)	HGARFPTSGA	ATRIKAGLSK	LQSvqnfTDP	KFDFIkSfTY	dLGtsDLVPF
T. pubescens	HGARFPTSGA	AkRIQTAVAK	LKAAsnyTDP	lLAFVtNyTY	sLGqDsLVeL
A. pediades	HGARFPTSGA	GTRIQAAVkK	LQSAktyTDP	RLDFLtNyTY	tLGhDDLVPF
P. lycii	HGARWPTSGA	rSRqvAAVAK	IQmArpfTDP	KYEFLnDfvY	kFGvADLLPF
Basidio	**HGARFPTSGA**	**ATRIQAAVAK**	**LQSA---TDP**	**KLDFL-N-TY**	**-LG-DDLVPF**

	101				150
P. involutus (*phyA1*)	GAaQSfDAGQ	EAFARYSkLV	SkNNLPFIRA	dGSDRVVDSA	TNWTAGFAsA
P. involutus (phyA2)	GAaQSfDAGl	EvFARYSkLV	SsDNLPFIRS	dGSDRVVDTA	TNWTAGFAsA
T. pubescens	GAtQSSEAGQ	EAFTRYSsLV	SaDELPFVRA	SGSDRVVATA	nNWTAGFAlA
A. pediades	GAlQSSQAGE	ETFqRYSfLV	SkENLPFVRA	SSSNRVVDSA	TNWTEGFSaA
P. lycii	GAnQShQTGt	DmYTRYStLf	egGDVPFVRA	AGdQRVVDSS	TNWTAGFGdA
Basidio	**GA-QSSQAGQ**	**EAFTRYS-LV**	**S-DNLPFVRA**	**SGSDRVVDSA**	**TNWTAGFA-A**

	151				200
P. involutus (phyA1)	ShNTvqPkLn	LILPQtGNDT	LEDNMCPaAG	DSDPQvNaWL	AVafPSITAR
P. involutus (phyA2)	SrNAiqPkLd	LILPQtGNDT	LEDNMCPaAG	ESDPQvDaWL	AsafPSVTAQ
T. pubescens	SsNSitPvLs	VIISEaGNDT	LDDNMCPaAG	DSDPQvNqWL	AqFAPPMTAR
A. pediades	ShHvlnPiLf	VILSEslNDT	LDDaMCPnAG	sSDPQtGiWt	SIYGTPIAnR
P. lycii	SgETvlPtLq	VVLqEeGNcT	LcNNMCPnEv	DGDest.tWL	GVFAPnITAR
Basidio	**S-NT--P-L-**	**VILSE-GNDT**	**LDDNMCP-AG**	**DSDPQ-N-WL**	**AVFAPPITAR**

	201				250
P. involutus (phyA1)	LNAAAPSvNL	TDtDAfNLvs	LCAFlTVSkE	kkSdFCtLFE	giPGsFeAFa
P. involutus (phyA2)	LNAAAPGANL	TDaDAfNLvs	LCPFmTVSkE	qkSdFCtLFE	giPGsFeAFa
T. pubescens	LNAGAPGANL	TDtDTyNLlt	LCPFETVAtE	rrSeFCDIYE	elQAE.dAFa
A. pediades	LNqqAPGANI	TAaDvsNLip	LCAFETIvkE	tpSpFCNLF.	.tPEEFaqFe
P. lycii	LNAAAPSANL	SDsDAltLmd	MCPFDTLSsG	naSpFCDLF.	.tAEEYvSYe
Basidio	**LNAAAPGANL**	**TD-DA-NL--**	**LCPFETVS-E**	**--S-FCDLFE**	**--PEEF-AF-**

	251				300
P. involutus (phyA1)	YgGDLDKFYG	TGYGQeLGPV	QGVGYVNELI	ARLTnsAVRD	NTQTNRTLDA
P. involutus (phyA2)	YaGDLDKFYG	TGYGQALGPV	QGVGYINELL	ARLTnsAVnD	NTQTNRTLDA
T. pubescens	YnADLDKFYG	TGYGQPLGPV	QGVGYINELI	ARLTaQnVsD	HTQTNsTLDS
A. pediades	YfGDLDKFYG	TGYGQPLGPV	QGVGYINELL	ARLTemPVRD	NTQTNRTLDS

P. lycii	YyyDLDKYYG	TGpGNALGPV	QGVGYVNELL	ARLTgQAVRD	ETQTNRTLDS
Basidio	**Y-GDLDKFYG**	**TGYGQPLGPV**	**QGVGYINELL**	**ARLT-QAVRD**	**NTQTNRTLDS**

	301				350
P. involutus (*phyA1*)	SPvTFPLNKT	FYADFSHDNl	MVAVFSAMGL	FrQPAPLsTS	vPNPwRTWrT
P. involutus (*phyA2*)	APdTFPLNKT	MYADFSHDNl	MVAVFSAMGL	FrQSAPLsTS	tPDPNRTWLT
T. pubescens	SPeTFPLNRT	LYADFSHDNQ	MVAIFSAMGL	FNQSAPLDPT	tPDPaRTFLv
A. pediades	SPlTFPLDRS	IYADLSHDNQ	MIAIFSAMGL	FNQSSPLDPS	fPNPKRTWVT
P. lycii	dPaTFPLNRT	FYADFSHDNt	MVPIFAALGL	FNaTA.LDPl	kPDeNRlWVd
Basidio	**SP-TFPLNRT**	**FYADFSHDNQ**	**MVAIFSAMGL**	**FNQSAPLDPS**	**-PDPNRTWVT**

	351				400
P. involutus (*phyA1*)	SsLVPFSGRM	VVERLsC..f	GT........	.......tkV	RVLVQDqVQP
P. involutus (*phyA2*)	SsVVPFSARM	aVERLsC..a	GT........	.......tkV	RVLVQDqVQP
T. pubescens	kKIVPFSARM	VVERLdC..g	GA........	.......qsV	RLLVNDAVQP
A. pediades	SRLtPFSARM	VtERLlCqrd	GTgsggpsri	mrngnvqtfV	RILVNDALQP
P. lycii	SKLVPFSGHM	tVEKLaC...	....	....sgkeaV	RVLVNDAVQP
Basidio	**SKLVPFSARM**	**VVERL-C---**	**GT--------**	**---------V**	**RVLVNDAVQP**

	401			441
P. involutus (phyA1)	LEFCGGDrNG	lCTLAkFVES	QtFARsDGaG	DFEKCFATSa ~
P. involutus (phyA2)	LEFCGGDqDG	lCALDkFVES	QaYARsGGaG	DFEKCLATTv ~
T. pubescens	LAFCGADtsG	vCTLDAFVES	QaYARNDGEG	DFEKCFAT~~ ~
A. pediades	LKFCGGDmDS	lCTLEAFVES	QkYAREDGQG	DFEKCFD~~~ ~
P. lycii	LEFCGG.vDG	vCeLsAFVES	QtYARENGQG	DFAKCgfvPs e
Basidio	**LEFCGGD-DG**	**-CTLDAFVES**	**Q-YAREDGQG**	**DFEKCFATP- -**

Figure 4

	1				50
A. terreus 9a1	KhsdCNSVDh	GYQCfPELSH	kWGlYAPYFS	LqDESPFPlD	VPeDCHITFV
A. terreus cbs	NhsdCtSVDr	GYQCfPELSH	kWGlYAPYFS	LqDESPFPlD	VPdDCHITFV
A. niger var. *awamori*	NqsTCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESAISPD	VPaGCRVTFa
A. niger NRRL3135	NqsSCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESvISPE	VPaGCRVTFa
A. fumigatus 13073	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
A. fumigatus 32722	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
A. fumigatus 58128	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
A. fumigatus 26906	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
A. fumigatus 32239	GSkACDTVEl	GYQCsPGtSH	LWGQYSPFFS	LEDElSVSSD	LPkDCRVTFV
E. nidulans	QNHSCNTaDG	GYQCfPNVSH	VWGQYSPYFS	IEQESAISeD	VPhGCeVTFV
T. thermophilus	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPqNCKITFV
T. lanuginosa	~~~~~~~~~~	~~~~nvDIAR	hWGQYSPFFS	LAEvSEISPA	VPkGCRVeFV
M. thermophila	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS	VPsElDaS..	IPdDCeVTFa
Basidio	xSxPxrxtAA	qLPipxQxqx	xWSPYSPYFP	VAxyxA....	pPaGCQIxqV
Consensus	NSHSCDTVDG	GYQC-PEISH	LWGQYSPFFS	LADESAISPD	VP-GCRVTFV
Fcp10	**NSHSCDTVDG**	**GYQCFPEISH**	**LWGQYSPFFS**	**LADESAISPD**	**VPKGCRVTFV**

	51				100
A. terreus 9a1	QVLARHGARs	PThSKTKaYA	AtIaAIQKSA	TaFpGKYAFL	QSYNYSLDSE
A. terreus cbs	QVLARHGARs	PTdSKTKaYA	AtIaAIQKNA	TaLpGKYAFL	KSYNYSMGSE
A. niger var. *awamori*	QVLSRHGARY	PTeSKGKKYS	ALIeEIQQNv	TtFDGKYAFL	KTYNYSLGAD
A. niger NRRL3135	QVLSRHGARY	PTdSKGKKYS	ALIeEIQQNA	TtFDGKYAFL	KTYNYSLGAD
A. fumigatus 13073	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 32722	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 58128	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 26906	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 32239	QVLSRHGARY	PTASKSKKYk	kLVtAIQKNA	TeFKGKFAFL	ETYNYTLGAD
E. nidulans	QVLSRHGARY	PTeSKSKaYS	GLIeAIQKNA	TsFwGQYAFL	ESYNYTLGAD
T. thermophilus	QLLSRHGARY	PTSSKTElYS	qLIsrIQKtA	TaYKGyYAFL	KdYrYqLGAN
T. lanuginosa	QVLSRHGARY	PTAhKSEvYA	ELLqrIQDtA	TeFKGDFAFL	RdYaYhLGAD
M. thermophila	QVLSRHGARa	PTlkRAasYv	DLIdrIHhGA	isYgPgYEFL	RTYDYTLGAD
Basidio	NIIqRHGARF	PTSGaAtRiq	AaVakLQsax	xxtDPKLDFL	xnxtYxLGxD
Consensus	QVLSRHGARY	PTSSKSKKYS	ALI-AIQKNA	T-FKGKYAFL	KTYNYTLGAD
Fcp10	**QVLSRHGARY**	**PTSSKSKKYS**	**ALIEAIQKNA**	**TAFKGKYAFL**	**KTYNYTLGAD**

	101				150
A. terreus 9a1	ELTPFGrNQL	rDlGaQFYeR	YNAL.TRhIn	PFVRATDAsR	VhESAEKFVE
A. terreus cbs	NLTPFGrNQL	qDlGaQFYRR	YDTL.TRhIn	PFVRAADSsR	VhESAEKFVE
A. niger var. *awamori*	DLTPFGEQEL	VNSGIKFYQR	YESL.TRnII	PFIRSSGSsR	VIASGEKFIE
A. niger NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESL.TRnIV	PFIRSSGSsR	VIASGKKFIE
A. fumigatus 13073	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus 32722	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus 58128	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus 26906	DLTAFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus 32239	DLTPFGEQQM	VNSGIKFYQK	YKAL.AgsVV	PFIRSSGSDR	VIASGEKFIE
E. nidulans	DLTiFGENQM	VDSGaKFYRR	YKnL.ARknt	PFIRASGSDR	VVASAEKFIN
T. thermophilus	DLTPFGENQM	IQlGIKFYnH	YKSL.ARnaV	PFVRCSGSDR	VIASGrlFIE
T. lanuginosa	NLTRFGEEQM	MESGrQFYHR	YREq.AReIV	PFVRAAGSAR	VIASAEfFnr
M. thermophila	ELTRtGQQQM	VNSGIKFYRR	YRAL.ARksI	PFVRTAGqDR	VVhSAENFtQ
Basidio	DLvPFGAxQs	sQAGqEaFtR	YsxLvSxdnL	PFVRASGSDR	VVDSAtNWtA
Consensus	DLTPFGEQQM	VNSGIKFYRR	YKAL-AR-IV	PFVRASGSDR	VIASAEKFIE
Fcp10	**DLTPFGEQQM**	**VNSGIKFYRR**	**YKAL.ARKIV**	**PFVRASGSDR**	**VIASAEKFIE**

	151				200
A. terreus 9a1	GFQTARqDDh	hAnphQPSPr	VDVaIPEGsA	YNNTLEHSLC	TAFEs...St
A. terreus cbs	GFQNARqGDP	hAnphQPSPr	VDVVIPEGtA	YNNTLEHSIC	TAFEa...St
A. niger var. *awamori*	GFQSTKLkDP	rAqpgQSSPk	IDVVISEAsS	sNNTLDpGtC	TvFEd...SE
A. niger NRRL3135	GFQSTKLkDP	rAqpgQSSPk	IDVVISEAsS	sNNTLDpGtC	TvFEd...SE
A. fumigatus 13073	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEa...SQ
A. fumigatus 32722	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEa...SQ
A. fumigatus 58128	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEa...SQ
A. fumigatus 26906	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEa...SQ
A. fumigatus 32239	GFQqANVADP	gAt.nRAAPV	ISVIIPESeT	YNNTLDHSVC	TnFEa...SE
E. nidulans	GFRkAQLhDh	g.s.gQATPV	VNVIIPEidG	FNNTLDHStC	vSFEn...dE
T. thermophilus	GFQSAKVlDP	hSdkhDAPPt	INVIIeEGpS	YNNTLDtGsC	PvFEd...Ss
T. lanuginosa	GFQdAKdrDP	rSnkdQAePV	INVIISEEtG	sNNTLDgltC	PAaEe...Ap
M. thermophila	GFHSAlLADR	gStvrPTlPy	dmVVIPETaG	aNNTLHNDLC	TAFEegPySt
Basidio	GFaxA.....	..sxntxxPx	LxVILSExg.	.NDTLDDNMC	......PxAG
Consensus	GFQSAKLADP	-A---QASPV	INVIIPEG-G	YNNTLDHGLC	TAFE--P-SE
Fcp10	**GFQSAKLADP**	**GANPHQASPV**	**INVIIPEGAG**	**YNNTLDHGLC**	**TAFEE...SE**

	201				250
A. terreus 9a1	VGDDavANFT	AVFAPAIaqR	LEAdLPGVQL	StDDVVNLMA	MCPFETVSlT
A. terreus cbs	VGDAaADNFT	AVFAPAIakR	LEAdLPGVQL	SADDVVNLMA	MCPFETVSlT
A. niger var. *awamori*	LADtVEANFT	AtFAPSIRqR	LEndLSGVtL	TDtEVtyLMD	MCSFDTIStS
A. niger NRRL3135	LADtVEANFT	AtFvPSIRqR	LEndLSGVtL	TDtEVtyLMD	MCSFDTIStS
A. fumigatus 13073	LGDEVAANFT	ALFAPdIRAR	aEkhLPGVtL	TDEDVVSLMD	MCSFDTVArT
A. fumigatus 32722	LGDEVAANFT	ALFAPdIRAR	aEkhLPGVtL	TDEDVVSLMD	MCSFDTVArT
A. fumigatus 58128	LGDEVAANFT	ALFAPdIRAR	aEkhLPGVtL	TDEDVVSLMD	MCSFDTVArT
A. fumigatus 26906	LGDEVAANFT	ALFAPdIRAR	aKkhLPGVtL	TDEDVVSLMD	MCSFDTVArT
A. fumigatus 32239	LGDEVEANFT	ALFAPAIRAR	IEkhLPGVQL	TDDDVVSLMD	MCSFDTVArT
E. nidulans	rADEIEANFT	AIMGPPIRkR	LEndLPGIKL	TNENVIyLMD	MCSFDTMArT
T. thermophilus	gGHDaQEKFA	kqFAPAIlEK	IKDhLPGVDL	AvsDVpyLMD	LCPFETLArn
T. lanuginosa	.DptqpAEFl	qVFGPRVlkK	ItkhMPGVNL	TlEDVplFMD	LCPFDTVGsd
M. thermophila	IGDDaQDtYl	StFAGPItAR	VNAnLPGaNL	TDADtVaLMD	LCPFETVAsS
Basidio	dSDpqxnxWl	AVFAPPItAR	LNAaaPGaNL	TDxDaxNLxx	LCPFETVS..
Consensus	LGDDVEANFT	AVFAPPIRAR	LEA-LPGVNL	TDEDVVNLMD	MCPFDTVA-T
Fcp10	**LGDDVEANFT**	**AVFAPPIRAR**	**LEAHLPGVNL**	**TDEDVVNLMD**	**MCPFDTVART**

	251				300
A. terreus 9a1	dD..Aht...	......LSPF	CDLFTa..tE	WtQYNYLlSL	dKYYGYGGGN
A. terreus cbs	dD..Aht...	......LSPF	CDLFTa..aE	WtQYNYLlSL	dKYYGYGGGN
A. niger var. *awamori*	Tv..DTK...	......LSPF	CDLFTH..dE	WiHYDYLQSL	kKYYGHGAGN
A. niger NRRL3135	Tv..DTK...	......LSPF	CDLFTH..dE	WiNYDYLQSL	kKYYGHGAGN
A. fumigatus 13073	SD..ASQ...	......LSPF	CQLFTH..nE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus 32722	SD..ASQ...	......LSPF	CQLFTH..nE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus 58128	SD..ASQ...	......LSPF	CQLFTH..nE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus 26906	SD..ASQ...	......LSPF	CQLFTH..nE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus 32239	AD..ASE...	......LSPF	CAIFTH..nE	WkKYDYLQSL	gKYYGYGAGN
E. nidulans	AH..GTE...	......LSPF	CAIFTE..kE	WlQYDYLQSL	sKYYGYGAGS
T. thermophilus	ht..DT....	......LSPF	CALsTQ..eE	WqaYDYYQSL	gKYYGnGGGN
T. lanuginosa	PvlfPrQ...	......LSPF	CHLFTa..dD	WmaYDYYyTL	dKYYSHGGGS
M. thermophila	SsdpATadag	ggngrpLSPF	CrLFSE..sE	WraYDYLQSV	gKWYGYGPGN
Basidio	....	...xexxSxF	CDLFexxpeE	FxaFxYxgdL	dKFYGtGyGQ
Consensus	SD--ATQ---	------LSPF	CDLFTH---E	W-QYDYLQSL	-KYYGYGAGN
Fcp10	**SD..ATQ...**	**......LSPF**	**CDLFTH..DE**	**WIQYDYLQSL**	**GKYYGYGAGN**

	301				350
A. terreus 9a1	PLGPvQGVGW	aNELMARLTR	A.PVHDHTCv	NNTLDASPAT	FPLNATLYAD
A. terreus cbs	PLGPvQGVGW	aNELIARLTR	S.PVHDHTCv	NNTLDANPAT	FPLNATLYAD
A. niger var. *awamori*	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS	NHTLDSNPAT	FPLNSTLYAD
A. niger NRRL3135	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS	NHTLDSSPAT	FPLNSTLYAD
A. fumigatus 13073	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus 32722	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus 58128	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus 26906	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus 32239	PLGPAQGIGF	tNELIARLTN	S.PVQDHTST	NsTLDSDPAT	FPLNATIYvD
E. nidulans	PLGPAQGIGF	tNELIARLTQ	S.PVQDNTST	NHTLDSNPAT	FPLDrkLYAD
T. thermophilus	PLGPAQGVGF	vNELIARMTH	S.PVQDYTTv	NHTLDSNPAT	FPLNATLYAD
T. lanuginosa	AFGPSRGVGF	vNELIARMTg	N1PVKDHTTv	NHTLDdNPET	FPLDAvLYAD
M. thermophila	PLGPTQGVGF	vNELLARLA.	GvPVRDgTST	NRTLDGDPrT	FPLGrPLYAD
Basidio	PLGPvQGVGY	iNELLARLTx	qa.VRDNTqT	NRTLDSSPxT	FPLNrTFYAD
Consensus	PLGPAQGVGF	-NELIARLTH	S-PVQDHTST	NHTLDSNPAT	FPLNATLYAD
Fcp10	**PLGPAQGVGF**	**VNELIARLTH**	**S.PVQDHTST**	**NHTLDSNPAT**	**FPLNATLYAD**

	351				400
A. terreus 9a1	FSHDSnLVSI	FWALGLYNGT	aPLSqTSVE.	.SvsQTDGYA	AAWTVPFAAR
A. terreus cbs	FSHDSnLVSI	FWALGLYNGT	kPLSqTTVE.	.ditrTDGYA	AAWTVPFAAR
A. niger var. *awamori*	FSHDNGIISI	LFALGLYNGT	kPLSTTTVE.	.NitQTDGFS	SAWTVPFASR
A. niger NRRL3135	FSHDNGIISI	LFALGLYNGT	kPLSTTTVE.	.NitQTDGFS	SAWTVPFASR
A. fumigatus 13073	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS	ASWvVPFGAR
A. fumigatus 32722	FSHDNSMVSI	FFALGLYNGT	gPLSrTSVE.	.SaKElDGYS	ASWvVPFGAR
A. fumigatus 58128	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS	ASWvVPFGAR
A. fumigatus 26906	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS	ASWvVPFGAR
A. fumigatus 32239	FSHDNGMIPI	FFAMGLYNGT	ePLSqTSeE.	.StKESNGYS	ASWAVPFGAR
E. nidulans	FSHDNSMISI	FFAMGLYNGT	qPLSmdSVE.	.SiQEmDGYA	ASWTVPFGAR
T. thermophilus	FSHDNTMtSI	FaALGLYNGT	akLSTTeIK.	.SiEETDGYS	AAWTVPFGGR
T. lanuginosa	FSHDNTMtGI	FsAMGLYNGT	kPLSTSkIQP	pTgAAADGYA	ASWTVPFAAR
M. thermophila	FSHDNdMMGV	LgALGaYDGv	pPLdkTA..R	rdpEElGGYA	ASWAVPFAAR
Basidio	FSHDNqMVAI	FsAMGLFNqS	aPLdPSxpDP	nrt.....Wv	TSklVPFSAR
Consensus	FSHDNTMVSI	FFALGLYNGT	-PLSTTSVEP	-S-EETDGYA	ASWTVPFAAR
Fcp10	**FSHDNTMVSI**	**FFALGLYNGT**	**KPLSTTSVE.**	**.SIEETDGYA**	**ASWTVPFAAR**

	401				450
A. terreus 9a1	AYVEMMQC..	ra........	.....EKEPL	VRVLVNDRVM	PLHGCPtDKL
A. terreus cbs	AYIEMMQC..	ra........	.....EKQPL	VRVLVNDRVM	PLHGCAVDNL
A. niger var. *awamori*	lYVEMMQC..	Qa........	.....EQEPL	VRVLVNDRVV	PLHGCPIDaL
A. niger NRRL3135	lYVEMMQC..	Qa........	.....EQEPL	VRVLVNDRVV	PLHGCPVDaL
A. fumigatus 13073	AYfEtMQC..	Ks........	.....EKEPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus 32722	AYfEtMQC..	Ks........	.....EKEPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus 58128	AYfEtMQC..	Ks........	.....EKESL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus 26906	AYfEtMQC..	Ks........	.....EKEPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus 32239	AYfEtMQC..	Ks........	.....EKEPL	VRaLINDRVV	PLHGCAVDKL
E. nidulans	AYfELMQC..	E.........	.....KKEPL	VRVLVNDRVV	PLHGCAVDKF
T. thermophilus	AYIEMMQC..	Dd........	.....sDEPV	VRVLVNDRVV	PLHGCEVDsL
T. lanuginosa	AYVELLRC..	Etetsseeee	EG...EDEPF	VRVLVNDRVV	PLHGCrVDRW
M. thermophila	iYVEkMRC..	sggggggggg	EGrqeKDEeM	VRVLVNDRVM	TLkGCGaDEr
Basidio	mvVErLxCxx	xgtxxxxxxx	xxxxxxxxxx	VRVLVNDaVq	PLEfCGgDxd
Consensus	AYVEMMQC--	E---------	EG---EKEPL	VRVLVNDRVV	PLHGCGVDKL
Fcp10	**AYVEMMQC..**	**EA........**	**.....EKEPL**	**VRVLVNDRVV**	**PLHGCGVDKL**

	451			482
A. terreus 9a1	GRCKrDAFVA	GLSFAQAG..	GNWADCF---	--
A. terreus cbs	GRCKrDDFVE	GLSFARAG..	GNWAECF---	--
A. niger var. *awamori*	GRCtrDsFVr	GLSFARSG..	GDWAECsA--	--
A. niger NRRL3135	GRCtrDsFVr	GLSFARSG..	GDWAECFA--	--
A. fumigatus 13073	GRCKlNDFVK	GLSWARSG..	GNWGECFS--	--
A. fumigatus 32722	GRCKlNDFVK	GLSWARSG..	GNWGECFS--	--
A. fumigatus 58128	GRCKlNDFVK	GLSWARSG..	GNWGECFS--	--
A. fumigatus 26906	GRCKlNDFVK	GLSWARSG..	GNWGECFS--	--
A. fumigatus 32239	GRCKlKDFVK	GLSWARSG..	GNSEQSFS--	--
E. nidulans	GRCtlDDWVE	GLNFARSG..	GNWKtCFTl-	--
T. thermophilus	GRCKrDDFVr	GLSFARqG..	GNWEGCYAas	e-
T. lanuginosa	GRCRrDEWIK	GLTFARqG..	GHWDrCF---	--
M. thermophila	GmCtlErFIE	SMAFARGN..	GKWDlCFA--	--
Basidio	GxCtlDAFVE	SqxYAReDgq	GDFEKCFAtp	xx
Consensus	GRCK-DDFVE	GLSFARSG--	GNWEECFA--	--
Fcp10	**GRCKRDDFVE**	**GLSFARSG..**	**GNWEECFA..**	**..**

Figure 5

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     CP-1
       EcoRI M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T   17
     TATATGAATTCATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCA
   1 ---------+---------+---------+---------+---------+---------+ 60
     ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT

      S  G  T  A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G  37
     CATCCGGTACCGCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG
  61 ---------+---------+---------+---------+---------+---------+ 120
     GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC
         CP-2
             CP-3.10
      Y  Q  C  F  P  E  I  S  H  L  W  G  Q  Y  S  P  F  F  S  L  57
     GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATTCTTCTCTT
 121 ---------+---------+---------+---------+---------+---------+ 180
     CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTAAGAAGAGAA

      A  D  E  S  A  I  S  P  D  V  P  K  G  C  R  V  T  F  V  Q  77
     TGGCTGACGAATCTGCTATTTCTCCAGACGTTCCAAAGGGTTGTAGAGTTACTTTCGTTC
 181 ---------+---------+---------+---------+---------+---------+ 240
     ACCGACTGCTTAGACGATAAAGAGGTCTGCAAGGTTTCCCGACATCTCAATGAAAGCAAG
                   CP-4.10
                       CP-5.10
      V  L  S  R  H  G  A  R  Y  P  T  S  S  K  S  K  K  Y  S  A  97
     AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTTCTTCTAAGTCTAAGAAGTACTCTG
 241 ---------+---------+---------+---------+---------+---------+ 300
     TTCAAAACAGATCTGTGCCACGATCTATGGGTTGAAGAAGATTCAGATTCTTCATGAGAC

      L  I  E  A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K 117
     CTTTGATTGAAGCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
 301 ---------+---------+---------+---------+---------+---------+ 360
     GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT
                                   CP-6
                                       CP-7.10
      T  Y  N  Y  T  L  G  A  D  D  L  T  P  F  G  E  Q  Q  M  V 137
     AGACTTACAACTACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAACAACAAATGG
 361 ---------+---------+---------+---------+---------+---------+ 420
     TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACC

      N  S  G  I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F 157
     TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT
 421 ---------+---------+---------+---------+---------+---------+ 480
     AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA
                                                 CP-8.10
                                                     CP-9.10
      V  R  A  S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F 177
     TCGTTAGAGCTTCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT
 481 ---------+---------+---------+---------+---------+---------+ 540
     AGCAATCTCGAAGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

      Q  S  A  K  L  A  D  P  G  A  N  P  H  Q  A  S  P  V  I  N 197
     TCCAATCTGCTAAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTA
 541 ---------+---------+---------+---------+---------+---------+ 600
     AGGTTAGACGATTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAAT
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                                                 CP-10.10
                                                      CP-11.10
       V  I  I  P  E  G  A  G  Y  N  N  T  L  D  H  G  L  C  T  A  217
      ACGTTATTATTCCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTG
 601  ---------+---------+---------+---------+---------+---------+ 660
      TGCAATAATAAGGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGAC

       F  E  E  S  E  L  G  D  D  V  E  A  N  F  T  A  V  F  A  P  237
      CTTTCGAAGAATCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTCGCTC
 661  ---------+---------+---------+---------+---------+---------+ 720
      GAAAGCTTCTTAGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAG
                                                            CP-12.10

       P  I  R  A  R  L  E  A  H  L  P  G  V  N  L  T  D  E  D  V  257
      CACCTATTAGAGCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACG
 721  ---------+---------+---------+---------+---------+---------+ 780
      GTGGATAATCTCGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGC

      CP-13.10
       V  N  L  M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  Q  277
      TTGTTAACTTGATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTC
 781  ---------+---------+---------+---------+---------+---------+ 840
      AACAATTGAACTACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAG

       L  S  P  F  C  D  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  297
      AATTGTCTCCATTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGC
 841  ---------+---------+---------+---------+---------+---------+ 900
      TTAACAGAGGTAAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACG
          CP-14.10
             CP-15.10
       S  L  G  K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  317
      AATCTTTGGGTAAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTG
 901  ---------+---------+---------+---------+---------+---------+ 960
      TTAGAAACCCATTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCAC

       G  F  V  N  E  L  I  A  R  L  T  H  S  P  V  Q  D  H  T  S  337
      TTGGTTTCGTTAACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTT
 961  ---------+---------+---------+---------+---------+---------+ 1020
      AACCAAAGCAATTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAA
                CP-16.10
                  CP-17.10
       T  N  H  T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A  357
      CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG
1021  ---------+---------+---------+---------+---------+---------+ 1080
      GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

       D  F  S  H  D  N  T  M  V  S  I  F  F  A  L  G  L  Y  N  G  377
      CTGACTTCTCTCACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACG
1081  ---------+---------+---------+---------+---------+---------+ 1140
      GACTGAAGAGAGTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGC
                                CP-18.10
                                   CP-19.10
       T  K  P  L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  A  A  397
      GTACTAAGCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACGCTG
1141  ---------+---------+---------+---------+---------+---------+ 1200
      CATGATTCGGTAACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGCGAC
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      S  W  T  V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  E  A  E 417
     CTTCTTGGACTGTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTG
1201 ---------+---------+---------+---------+---------+---------+ 1260
     GAAGAACCTGACAAGGTAAGCCACGATCTCGAATGCAACTTTACTACGTTACACTTCGAC
                                             CP-20.10
                                                  CP-21.10

      K  E  P  L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  G 437
     AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC

      V  D  K  L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R 457
     GTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA
1321 ---------+---------+---------+---------+---------+---------+ 1380
     CACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
                                                     CP-22.10

      S  G  G  N  W  E  E  C  F  A  *  Eco RI        467
     GATCTGGTGGTAACTGGGAAGAATGTTTCGCTTAAGAATTCATATA
1381 ---------+---------+---------+---------+------ 1426
     CTAGACCACCATTGACCCTTCTTACAAAGCGAATTCTTAAGTATAT
```

Figure 6

	1				50
P. involutus (phyA1)	~~~~~~~~~~	~FPipeseqR	nWSPYSPYFP	LAEykA....	pPaGCQInqV
P. involutus (phyA2)	~~~~~~~~~~	~FsipeseqR	nWSPYSPYFP	LAEykA....	pPaGCeInqV
T. pubescens	~~~~~~~~~~	~LDvtRDVqQ	sWSmYSPYFP	aAtyvA....	pPaSCQInqV
A. pediades	~~~~~~~~~~	~pffpPQIqD	sWAaYTPYYP	VqAyTP....	pPKDCKITqV
P. lycii	~~~~~~~~~~	~LPipAQnTs	nWGPYdPFFP	VEpyAA....	pPEGCtVTqV
A. terreus 9a1	KhsdCNSVDh	GYQCfPELSH	kWGlYAPYFS	LqDESPFPlD	VPEDCHITFV
A. terreus cbs	NhsdCtSVDr	GYQCfPELSH	kWGlYAPYFS	LqDESPFPlD	VPDDCHITFV
A. niger var. *awamori*	NqsTCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESAISPD	VPaGCRVTFa
A. niger T213	NqsSCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESvISPD	VPaGCRVTFa
A. niger NRRL3135	NqsSCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESvISPE	VPaGCRVTFa
A. fumigatus ATCC13073	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPKDCRITLV
A. fumigatus ATCC32722	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPKDCRITLV
A. fumigatus ATCC58128	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPKDCRITLV
A. fumigatus ATCC26906	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPKDCRITLV
A. fumigatus ATCC32239	GSkACDTVEl	GYQCsPGtSH	LWGQYSPFFS	LEDElSVSSD	LPKDCRVTFV
E. nidulans	QNHSCNTaDg	GYQCfPNVSH	VWGQYSPYFS	IEQESAISeD	VPhGCeVTFV
T. thermophilus	DSHSCNTVEg	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPQNCKITFV
T. lanuginosa	~~~~~~~~~~	~~~~nvDIAR	hWGQYSPFFS	LAEvSEISPA	VPKGCRVeFV
M. thermophila	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS	VPsElDaS..	IPDDCeVTFa
Consensus Seq. 11	**NSHSCDTVD-**	**GYQC-PEISH**	**LWGQYSPFFS**	**LADESAISPD**	**VPKGCRVTFV**

	51				100
P. involutus (phyA1)	NIIqRHGARF	PTSGaTtRik	AgLtKLQgvq	nftDAKFnFI	KSFKYdLGns
P. involutus (phyA2)	NIIqRHGARF	PTSGaAtRik	AgLsKLQsvq	nftDPKFDFI	KSFtYdLGTs
T. pubescens	HIIqRHGARF	PTSGaAKRiq	TaVAKLKaaS	nytDPlLAFV	tnYtYSLGqD
A. pediades	NIIqRHGARF	PTSGaGtRiq	AaVKKLQsak	TytDPRLDFL	tnYtYTLGhD
P. lycii	NLIqRHGARW	PTSGarsRqv	AaVAKIQmar	PftDPKYEFL	NdFvYkFGvA
A. terreus 9a1	QVLARHGARs	PThSKTKaYA	AtIAaIQKSA	TaFpGKYAFL	QSYNYSLDSE
A. terreus cbs	QVLARHGARs	PTdSKTKaYA	AtIAaIQKNA	TaLpGKYAFL	KSYNYSMGSE
A. niger var. *awamori*	QVLSRHGARY	PTeSKGKKYS	ALIEeIQQNv	TtFDGKYAFL	KTYNYSLGAD
A. niger T213	QVLSRHGARY	PTeSKGKKYS	ALIEeIQQNv	TtFDGKYAFL	KTYNYSLGAD
A. niger NRRL3135	QVLSRHGARY	PTdSKGKKYS	ALIEeIQQNA	TtFDGKYAFL	KTYNYSLGAD
A. fumigatus ATCC13073	QVLSRHGARY	PTSSKSKKYk	kLVtaIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus ATCC32722	QVLSRHGARY	PTSSKSKKYk	kLVtaIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus ATCC58128	QVLSRHGARY	PTSSKSKKYk	kLVtaIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus ATCC26906	QVLSRHGARY	PTSSKSKKYk	kLVtaIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus ATCC32239	QVLSRHGARY	PTASKSKKYk	kLVtaIQKNA	TeFKGKFAFL	ETYNYTLGAD
E. nidulans	QVLSRHGARY	PTeSKSKaYS	GLIEaIQKNA	TsFwGQYAFL	ESYNYTLGAD
T. thermophilus	QLLSRHGARY	PTSSKTElYS	qLIsRIQKtA	TaYKGyYAFL	KdYrYqLGAN
T. lanuginosa	QVLSRHGARY	PTAhKSEvYA	ELLQRIQDtA	TeFKGDFAFL	RdYaYhLGAD
M. thermophila	QVLSRHGARa	PTlkRAasYv	DLIDRIHhGA	isYgPgYEFL	RTYDYTLGAD
Consensus Seq. 11	**QVLSRHGARY**	**PTSSKSKKYS**	**ALIERIQKNA**	**T-FKGKYAFL**	**KTYNYTLGAD**

	101				150
P. involutus (*phyA1*)	DLvPFGAaQs	fDAGqEaFaR	YskLvSKNnL	PFIRAdGSDR	VVDSAtNWtA
P. involutus (*phyA2*)	DLvPFGAaQs	fDAGLEvFaR	YskLvSsDnL	PFIRSdGSDR	VVDTAtNWtA
T. pubescens	sLveLGAtQs	sEAGqEaFtR	YsSLvSaDeL	PFVRASGSDR	VVATANNWtA
A. pediades	DLvPFGAlQs	sQAGeEtFQR	YsfLvSKEnL	PFVRASSSNR	VVDSAtNWtE
P. lycii	DLlPFGANQs	hQTGtDMYtR	YsTLfEgGdV	PFVRAAGdQR	VVDSStNWtA
A. terreus 9a1	ELTPFGrNQL	rDlGaQFYeR	YNAL.TRHIn	PFVRATDAsR	VhESAEKFVE
A. terreus cbs	NLTPFGrNQL	qDlGaQFYRR	YDTL.TRHIn	PFVRAADSsR	VhESAEKFVE
A. niger var. *awamori*	DLTPFGEQEL	VNSGIKFYQR	YESL.TRNII	PFIRSSGSsR	VIASGEKFIE
A. niger T213	DLTPFGEQEL	VNSGIKFYQR	YESL.TRNII	PFIRSSGSsR	VIASGEKFIE
A. niger NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESL.TRNIV	PFIRSSGSsR	VIASGKKFIE
A. fumigatus ATCC13073	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus ATCC32722	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus ATCC58128	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus ATCC26906	DLTAFGEQQL	VNSGIKFYQR	YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus ATCC32239	DLTPFGEQQM	VNSGIKFYQK	YKAL.AgSVV	PFIRSSGSDR	VIASGEKFIE
E. nidulans	DLTiFGENQM	VDSGaKFYRR	YKnL.ARKnt	PFIRASGSDR	VVASAEKFIN
T. thermophilus	DLTPFGENQM	IQlGIKFYnH	YKSL.ARNaV	PFVRCSGSDR	VIASGrlFIE
T. lanuginosa	NLTRFGEEQM	MESGrQFYHR	YREq.AREIV	PFVRAAGSAR	VIASAEfFnr
M. thermophila	ELTRtGQQQM	VNSGIKFYRR	YRAL.ARKsI	PFVRTAGqDR	VVhSAENFtQ
Consensus Seq. 11	**DLTPFGENQM**	**VNSGIKFYRR**	**YKAL-ARNIV**	**PFVRASGSDR**	**VIASAEKFIE**

	151				200
P. involutus (*phyA1*)	GFaSA.....	..shNtvqPk	LNLILPQ..T	gNDTLEDNMC	PAaGD.....
P. involutus (*phyA2*)	GFaSA.....	..srNaiqPk	LDLILPQ..T	gNDTLEDNMC	PAaGE.....
T. pubescens	GFalA.....	..ssNsiTPV	LSVIISE..A	gNDTLDDNMC	PAaGD.....
A. pediades	GFsAA.....	..shHvlNPI	LfVILSE..S	LNDTLDDAMC	PnaGs.....
P. lycii	GFgdA.....	..sgEtvlPt	LQVVLQE..E	gNcTLcNNMC	PnevD.....
A. terreus 9a1	GFQTARqDDh	hAnpHQPSPr	VDVaIPEGSA	YNNTLEHSLC	TAFEs...ST
A. terreus cbs	GFQNARqGDP	hAnpHQPSPr	VDVVIPEGTA	YNNTLEHSIC	TAFEA...ST
A. niger var. *awamori*	GFQSTKLkDP	rAqpgQSSPk	IDVVISEASS	sNNTLDpGtC	TvFED...Se
A. niger T213	GFQSTKLkDP	rAqpgQSSPk	IDVVISEASS	sNNTLDpGtC	TvFED...Se
A. niger NRRL3135	GFQSTKLkDP	rAqpgQSSPk	IDVVISEASS	sNNTLDpGtC	TvFED...Se
A. fumigatus ATCC13073	GFQqAKLADP	gAt.NRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEA...Sq
A. fumigatus ATCC32722	GFQqAKLADP	gAt.NRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEA...Sq
A. fumigatus ATCC58128	GFQqAKLADP	gAt.NRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEA...Sq
A. fumigatus ATCC26906	GFQqAKLADP	gAt.NRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEA...Sq
A. fumigatus ATCC32239	GFQqANVADP	gAt.NRAAPV	ISVIIPESeT	YNNTLDHSVC	TnFEA...Se
E. nidulans	GFRkAQLhDh	g.s.gQATPV	VNVIIPEidG	FNNTLDHStC	vSFEN...de
T. thermophilus	GFQSAKVlDP	hSdkHDAPPt	INVIIeEGPS	YNNTLDtGsC	PvFED...SS
T. lanuginosa	GFQdAKdrDP	rSnkDQAePV	INVIISEETG	sNNTLDgltC	PAaEE...AP
M. thermophila	GFHSAlLADR	gStvRPTlPy	dmVVIPETAG	aNNTLHNDLC	TAFEEgpyST
Consensus Seq. 11	**GFQSAKLADP**	**-A--HQASPV**	**INVIIPEGSG**	**YNNTLDHGLC**	**TAFED---ST**

	201				250
P. involutus (phyA1)	.SDpqvnaWl	AVafPSItAR	LNAaaPSVNL	TDtDafNLVs	LCAFlTVSK.
P. involutus (phyA2)	.SDpqvDaWl	AsafPSVtAQ	LNAaaPGaNL	TDADafNLVs	LCPFmTVSK.
T. pubescens	.SDpqvnQWl	AqFAPPMtAR	LNAgaPGaNL	TDtDtyNLLt	LCPFETVAt.
A. pediades	.SDpqtGiWT	SIYGTPIanR	LNqqaPGaNI	TAADVsNLIp	LCAFETIvK.
P. lycii	.GDESt.tWl	GVFAPnItAR	LNAaaPSaNL	SDsDaLtLMD	MCPFDTLSs.
A. terreus 9a1	VGDDAvANFT	AVFAPAIaqR	LEAdLPGVQL	StDDVVNLMA	MCPFETVSlT
A. terreus cbs	VGDAAADNFT	AVFAPAIakR	LEAdLPGVQL	SADDVVNLMA	MCPFETVSlT
A. niger var. *awamori*	LADtvEANFT	AtFAPSIRqR	LEndLSGVtL	TDtEVtyLMD	MCSFDTIStS
A. niger T213	LADtvEANFT	AtFAPSIRqR	LEndLSGVtL	TDtEVtyLMD	MCSFDTIStS
A. niger NRRL3135	LADtvEANFT	AtFvPSIRqR	LEndLSGVtL	TDtEVtyLMD	MCSFDTIStS
A. fumigatus ATCC13073	LGDEvAANFT	ALFAPdIRAR	aEkhLPGVtL	TDEDVVSLMD	MCSFDTVART
A. fumigatus ATCC32722	LGDEvAANFT	ALFAPdIRAR	aEkhLPGVtL	TDEDVVSLMD	MCSFDTVART
A. fumigatus ATCC58128	LGDEvAANFT	ALFAPdIRAR	aEkhLPGVtL	TDEDVVSLMD	MCSFDTVART
A. fumigatus ATCC26906	LGDEvAANFT	ALFAPdIRAR	aKkhLPGVtL	TDEDVVSLMD	MCSFDTVART
A. fumigatus ATCC32239	LGDEvEANFT	ALFAPAIRAR	IEkhLPGVQL	TDDDVVSLMD	MCSFDTVART
E. nidulans	rADEiEANFT	AIMGPPIRkR	LEndLPGIKL	TNENVIyLMD	MCSFDTMART
T. thermophilus	gGHDAQEKFA	kqFAPAIlEK	IKDhLPGVDL	AvsDVpyLMD	LCPFETLARn
T. lanuginosa	.DptqpAEFl	qVFGPRVlkK	ItkhMPGVNL	TlEDVplFMD	LCPFDTVGsd
M. thermophila	IGDDAQDtYl	StFAGPItAR	VNAnLPGaNL	TDADtVaLMD	LCPFETVAsS
Consensus Seq. 11	**LGDDAEANFT**	**AVFAPPIRAR**	**LEA-LPGVNL**	**TDEDVVNLMD**	**MCPFDTVART**

	251				300
P. involutus (phyA1)	....	....ekkSdF	CtLFegiPGs	FeaFAYggdL	dKFYGtGyGQ
P. involutus (phyA2)	....	....eqkSdF	CtLFegiPGs	FeaFAYagdL	dKFYGtGyGQ
T. pubescens	....	....errSeF	CDIYeelqAE	.daFAYnadL	dKFYGtGyGQ
A. pediades	....	....etpSPF	CNLF..TPEE	FaQFEYFgdL	dKFYGtGyGQ
P. lycii	....	....gnaSPF	CDLF..TAEE	YvsYEYYydL	dKYYGtGPGN
A. terreus 9a1	dD..Aht...	......LSPF	CDLF..TAtE	WtQYNYLlSL	dKYYGYGGGN
A. terreus cbs	dD..Aht...	......LSPF	CDLF..TAAE	WtQYNYLlSL	dKYYGYGGGN
A. niger var. *awamori*	Tv..DTK...	......LSPF	CDLF..ThDE	WiHYDYLQSL	kKYYGHGAGN
A. niger T213	Tv..DTK...	......LSPF	CDLF..ThDE	WiHYDYLRSL	kKYYGHGAGN
A. niger NRRL3135	Tv..DTK...	......LSPF	CDLF..ThDE	WiNYDYLQSL	kKYYGHGAGN
A. fumigatus ATCC13073	SD..ASQ...	......LSPF	CQLF..ThNE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus ATCC32722	SD..ASQ...	......LSPF	CQLF..ThNE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus ATCC58128	SD..ASQ...	......LSPF	CQLF..ThNE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus ATCC26906	SD..ASQ...	......LSPF	CQLF..ThNE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus ATCC32239	AD..ASE...	......LSPF	CAIF..ThNE	WkKYDYLQSL	gKYYGYGAGN
E. nidulans	AH..GTE...	......LSPF	CAIF..TEKE	WlQYDYLQSL	sKYYGYGAGS
T. thermophilus	ht..DT....	......LSPF	CALs..TqEE	WqaYDYYQSL	gKYYGnGGGN
T. lanuginosa	PvlfPrQ...	......LSPF	CHLF..TADD	WmaYDYYyTL	dKYYSHGGGS
M. thermophila	SsdpATadag	ggngrpLSPF	CrLF..SEsE	WraYDYLQSV	gKWYGYGPGN
Consensus Seq. 11	**SD--ATQ---**	**------LSPF**	**CDLF--TADE**	**W-QYDYLQSL**	**-KYYGYGAGN**

	301				350
P. involutus (phyA1)	eLGPvQGVGY	vNELIARLTN	S.AVRDNTqT	NRTLDASPvT	FPLNkTFYAD
P. involutus (phyA2)	ALGPvQGVGY	iNELLARLTN	S.AVNDNTqT	NRTLDAaPDT	FPLNkTMYAD
T. pubescens	PLGPvQGVGY	iNELIARLTa	q.nVsDHTqT	NsTLDSSPET	FPLNrTLYAD
A. pediades	PLGPvQGVGY	iNELLARLTE	m.PVRDNTqT	NRTLDSSPlT	FPLDrSIYAD
P. lycii	ALGPvQGVGY	vNELLARLTg	q.AVRDETqT	NRTLDSDPAT	FPLNrTFYAD
A. terreus 9a1	PLGPvQGVGW	aNELMARLTR	A.PVHDHTCv	NNTLDASPAT	FPLNATLYAD
A. terreus cbs	PLGPvQGVGW	aNELIARLTR	S.PVHDHTCv	NNTLDANPAT	FPLNATLYAD
A. niger var. *awamori*	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS	NHTLDSNPAT	FPLNSTLYAD
A. niger T213	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS	NHTLDSNPAT	FPLNSTLYAD
A. niger NRRL3135	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS	NHTLDSSPAT	FPLNSTLYAD
A. fumigatus ATCC13073	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus ATCC32722	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus ATCC58128	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus ATCC26906	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus ATCC32239	PLGPAQGIGF	tNELIARLTN	S.PVQDHTST	NsTLDSDPAT	FPLNATIYvD
E. nidulans	PLGPAQGIGF	tNELIARLTQ	S.PVQDNTST	NHTLDSNPAT	FPLDrkLYAD
T. thermophilus	PLGPAQGVGF	vNELIARMTH	S.PVQDYTTv	NHTLDSNPAT	FPLNATLYAD
T. lanuginosa	AFGPSRGVGF	vNELIARMTg	NlPVKDHTTv	NHTLDdNPET	FPLDAvLYAD
M. thermophila	PLGPTQGVGF	vNELLARLA.	GvPVRDgTST	NRTLDGDPrT	FPLGrPLYAD
Consensus Seq. 11	**PLGPAQGVGF**	**-NELIARLTH**	**S-PVQDHTST**	**NHTLDSNPAT**	**FPLNATLYAD**

	351				400
P. involutus (phyA1)	FSHDNlMVAV	FsAMGLFrqP	aPLSTSvpNP	wrt.....Wr	TSSlVPFSGR
P. involutus (phyA2)	FSHDNlMVAV	FsAMGLFrqS	aPLSTSTpDP	nrt.....Wl	TSSvVPFSAR
T. pubescens	FSHDNqMVAI	FsAMGLFNqS	aPLdPTTpDP	art.....Fl	vkkiVPFSAR
A. pediades	LSHDNqMIAI	FsAMGLFNqS	sPLdPSfpNP	krt.....Wv	TSRltPFSAR
P. lycii	FSHDNTMVPI	FaALGLFNAT	a.LdPlkpDe	nrl.....Wv	DSklVPFSGH
A. terreus 9a1	FSHDSnLVSI	FWALGLYNGT	aPLSqTSVES	Vs..QTDGYA	AAWTVPFAAR
A. terreus cbs	FSHDSnLVSI	FWALGLYNGT	KPLSqTTVEd	It..rTDGYA	AAWTVPFAAR
A. niger var. *awamori*	FSHDNGIISI	LFALGLYNGT	KPLSTTTVEN	It..QTDGFS	SAWTVPFASR
A. niger T213	FSHDNGIISI	LFALGLYNGT	KPLSTTTVEN	It..QTDGFS	SAWTVPFASR
A. niger NRRL3135	FSHDNGIISI	LFALGLYNGT	KPLSTTTVEN	It..QTDGFS	SAWTVPFASR
A. fumigatus ATCC13073	FSHDNSMVSI	FFALGLYNGT	EPLSrTSVES	ak..ElDGYS	ASWvVPFGAR
A. fumigatus ATCC32722	FSHDNSMVSI	FFALGLYNGT	gPLSrTSVES	ak..ElDGYS	ASWvVPFGAR
A. fumigatus ATCC58128	FSHDNSMVSI	FFALGLYNGT	EPLSrTSVES	ak..ElDGYS	ASWvVPFGAR
A. fumigatus ATCC26906	FSHDNSMVSI	FFALGLYNGT	EPLSrTSVES	ak..ElDGYS	ASWvVPFGAR
A. fumigatus ATCC32239	FSHDNGMIPI	FFAMGLYNGT	EPLSqTSeES	tk..ESNGYS	ASWAVPFGAR
E. nidulans	FSHDNSMISI	FFAMGLYNGT	QPLSmdSVES	Iq..EmDGYA	ASWTVPFGAR
T. thermophilus	FSHDNTMtSI	FaALGLYNGT	akLSTTeIKS	Ie..ETDGYS	AAWTVPFGGR
T. lanuginosa	FSHDNTMtGI	FsAMGLYNGT	KPLSTSkIQP	ptgaAADGYA	ASWTVPFAAR
M. thermophila	FSHDNdMMGV	LgALGaYDGv	pPLdkTArrd	..peElGGYA	ASWAVPFAAR
Consensus Seq. 11	**FSHDNTMVSI**	**FFALGLYNGT**	**KPLSTTSVES**	**I---ETDGYA**	**ASWTVPFAAR**

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                         401                                                450
P. involutus (phyA1)     mvVErLsC.. fGt....... ........Tk VRVLVQDQVq PLEfCGgDRn
P. involutus (phyA2)     maVErLsC.. AGt....... ........Tk VRVLVQDQVq PLEfCGgDQd
T. pubescens             mvVErLDC.. GGa....... ........Qs VRLLVNDaVq PLafCGaDts
A. pediades              mvtErLlCQr DGtGsGGpsr imrNgnvQTF VRILVNDaLq PLkfCGgDmd
P. lycii                 mtVEkLaC.. .......... .....sgKea VRVLVNDaVq PLEfCGg.vd
A. terreus 9a1            AYVEMMQCrA .......... ..EK...EPL VRVLVNDRVM PLHGCPtDKL
A. terreus cbs           AYIEMMQCrA .......... ..EK...QPL VRVLVNDRVM PLHGCAVDNL
A. niger var. awamori    lYVEMMQCQA .......... ..EQ...EPL VRVLVNDRVV PLHGCPIDaL
A. niger T213            lYVEMMQCQA .......... ..EQ...EPL VRVLVNDRVV PLHGCPIDaL
A. niger NRRL3135        lYVEMMQCQA .......... ..EQ...EPL VRVLVNDRVV PLHGCPVDaL
A. fumigatus ATCC13073   AYfEtMQCKS .......... ..EK...EPL VRaLINDRVV PLHGCDVDKL
A. fumigatus ATCC32722   AYfEtMQCKS .......... ..EK...EPL VRaLINDRVV PLHGCDVDKL
A. fumigatus ATCC58128   AYfEtMQCKS .......... ..EK...ESL VRaLINDRVV PLHGCDVDKL
A. fumigatus ATCC26906   AYfEtMQCKS .......... ..EK...EPL VRaLINDRVV PLHGCDVDKL
A. fumigatus ATCC32239   AYfEtMQCKS .......... ..EK...EPL VRaLINDRVV PLHGCAVDKL
E. nidulans              AYfELMQCE. .......... ..KK...EPL VRVLVNDRVV PLHGCAVDKF
T. thermophilus          AYIEMMQCDD .......... ..sD...EPV VRVLVNDRVV PLHGCEVDsL
T. lanuginosa            AYVELLRCET ETsSeEEeEG ..ED...EPF VRVLVNDRVV PLHGCrVDRW
M. thermophila           iYVEkMRCsG GGgGgGGgEG ..rQekdEeM VRVLVNDRVM TLkGCGaDEr

Consensus Seq. 11        AYVEMMQCEA GG-G-GG-EG --EK---EPL VRVLVNDRVV PLHGCGVDKL
```

```
                         451                              482
P. involutus (phyA1)     GlCtLAKFVE SqTFARSDga GDFEKCFAts a~
P. involutus (phyA2)     GlCaLDKFVE SqAYARSGga GDFEKCLAtt v~
T. pubescens             GvCtLDAFVE SqAYARNDge GDFEKCFAt~ ~~
A. pediades              SlCtLEAFVE SqkYAReDgq GDFEKCFD~~ ~~
P. lycii                 GvCELsAFVE SqTYAReNgq GDFAKCgfvp se
A. terreus 9a1            GRCKrDAFVA GLSFAQAG.. GNWADCF~~~ ~~
A. terreus cbs           GRCKrDDFVE GLSFARAG.. GNWAECF~~~ ~~
A. niger var. awamori    GRCtrDsFVr GLSFARSG.. GDWAECsA~~ ~~
A. niger T213            GRCtrDsFVr GLSFARSG.. GDWAECFA~~ ~~
A. niger NRRL3135        GRCtrDsFVr GLSFARSG.. GDWAECFA-- ~~
A. fumigatus ATCC13073   GRCKLNDFVK GLSWARSG.. GNWGECFS~~ --
A. fumigatus ATCC32722   GRCKLNDFVK GLSWARSG.. GNWGECFS~~ --
A. fumigatus ATCC58128   GRCKLNDFVK GLSWARSG.. GNWGECFS~~ ~~
A. fumigatus ATCC26906   GRCKLNDFVK GLSWARSG.. GNWGECFS~~ ~~
A. fumigatus ATCC32239   GRCKLKDFVK GLSWARSG.. GNSEQSFS~~ ~~
E. nidulans              GRCtLDDWVE GLNFARSG.. GNWktCFTl~ ~~
T. thermophilus          GRCKrDDFVr GLSFARqG.. GNWEGCYAas e~
T. lanuginosa            GRCRrDEWIK GLTFARqG.. GHWDrCF~~~ ~~
M. thermophila           GmCtLErFIE SMAFARGN.. GKWDlCFA~~ ~~

Consensus Seq. 11        GRCKLDDFVE GLSFARSG-- GNWAECFA-- --
```

Figure 7

```
     M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T     20
     ATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCACATCCGGTACC
  1  ---------+---------+---------+---------+---------+---------+   60
     TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

     A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G  Y  Q  C     40
     GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
 61  ---------+---------+---------+---------+---------+---------+  120
     CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

     F  P  E  I  S  H  L  W  G  T  Y  S  P  Y  F  S  L  A  D  E     60
     TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA
121  ---------+---------+---------+---------+---------+---------+  180
     AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT

     S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q  V  L  S     80
     TCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTCAAGTTTTGTCT
187  ---------+---------+---------+---------+---------+---------+  240
     AGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAGTTCAAAACAGA

     R  H  G  A  R  Y  P  T  S  S  A  S  K  A  Y  S  A  L  I  E    100
     AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA
241  ---------+---------+---------+---------+---------+---------+  300
     TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAAACTAACTT

     A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K  T  Y  N    120
     GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC
301  ---------+---------+---------+---------+---------+---------+  360
     CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

     Y  T  L  G  A  D  D  L  T  P  F  G  E  N  Q  M  V  N  S  G    140
     TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGGTTAACTCTGGT
361  ---------+---------+---------+---------+---------+---------+  420
     ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA

     I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F  I  R  A    160
     ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT
421  ---------+---------+---------+---------+---------+---------+  480
     TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

     S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F  Q  S  A    180
     TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT
481  ---------+---------+---------+---------+---------+---------+  540
     AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

     K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  N  V  I  I    200
     AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT
541  ---------+---------+---------+---------+---------+---------+  600
     TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAATTGCACTAGTAA

     P  E  G  S  G  Y  N  N  T  L  D  H  G  T  C  T  A  F  E  D    220
     CCAGAAGGATCCGGTTACAACAACACTTTGGACCACGGTACTTGTACTGCTTTCGAAGAC
601  ---------+---------+---------+---------+---------+---------+  660
     GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGACGAAAGCTTCTG
```

```
      S  E  L  G  D  D  V  E  A  N  F  T  A  L  F  A  P  A  I  R    240
     TCTGAATTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTCGCTCCAGCTATTAGA
 661 ---------+---------+---------+---------+---------+---------+ 720
     AGACTTAATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTCGATAATCT

      A  R  L  E  A  D  L  P  G  V  T  L  T  D  E  D  V  V  Y  L    260
     GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG
 721 ---------+---------+---------+---------+---------+---------+ 780
     CGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGCTTCTGCAACAAATGAAC

      M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  E  L  S  P    280
     ATGGACATGTGTCCATTCGACACTGTCGCTAGAACTTCTGACGCTACTGAATTGTCTCCA
 781 ---------+---------+---------+---------+---------+---------+ 840
     TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT

      F  C  A  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  S  L  G    300
     TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT
 841 ---------+---------+---------+---------+---------+---------+ 900
     AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA

      K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  G  F  A    320
     AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGCT
 901 ---------+---------+---------+---------+---------+---------+ 960
     TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCGA

      N  E  L  I  A  R  L  T  H  S  P  V  Q  D  H  T  S  T  N  H    340
     AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC
 961 ---------+---------+---------+---------+---------+---------+ 1020
     TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

      T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A  D  F  S    360
     ACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACGCTGACTTCTCT
1021 ---------+---------+---------+---------+---------+---------+ 1080
     TGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

      H  D  N  T  M  I  S  I  F  F  A  L  G  L  Y  N  G  T  K  P    380
     CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACCAAGCCA
1081 ---------+---------+---------+---------+---------+---------+ 1140
     GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGGTTCGGT

      L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A  S  W  T    400
     TTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTGCTTCTTGGACT
1141 ---------+---------+---------+---------+---------+---------+ 1200
     AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

      V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  Q  A  E  K  E  P    420
     GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA
1201 ---------+---------+---------+---------+---------+---------+ 1260
     CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTCGACTTTTCCTTGGT

      L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A  V  D  K    440
     TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACTGTTC
```

```
      L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G    460
      TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321  ---------+---------+---------+---------+---------+---------+ 1380
      AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

      N  W  A  E  C  F  A  *    467
      AACTGGGCTGAATGTTTCGCTTAA
1381  ---------+---------+---- 1410
      TTGACCCGACTTACAAAGCGAATT
```

Figure 8

```
        M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T     20
        ATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCACATCCGGTACC
      1 ---------+---------+---------+---------+---------+---------+   60
        TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

        A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G  Y  Q  C     40
        GCCTTGGGTCCTCGTGGTAACTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
     61 ---------+---------+---------+---------+---------+---------+  120
        CGGAACCCAGGAGCACCATTGAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

A       F  P  E  I  S  H  L  W  G  T  Y  S  P  F  F  S  L  A  D  E     60
        TTCCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTTGGCTGACGAA
    121 ---------+---------+---------+---------+---------+---------+  180
        AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT

        S  A  I  S  P  D  V  P  K  G  C  R  V  T  F  V  Q  V  L  S     80
        TCTGCTATTTCTCCAGACGTTCCAAAGGGTTGTAGAGTTACTTTCGTTCAAGTTTTGTCT
    181 ---------+---------+---------+---------+---------+---------+  240
        AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA

        R  H  G  A  R  Y  P  T  S  S  A  S  K  A  Y  S  A  L  I  E    100
        AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCGTACTCTGCTTTGATTGAA
    241 ---------+---------+---------+---------+---------+---------+  300
        TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGCATGAGACGAAACTAACTT

        A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K  T  Y  N    120
        GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC
    301 ---------+---------+---------+---------+---------+---------+  360
        CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

A       Y  T  L  G  A  D  D  L  T  P  F  G  E  Q  Q  M  V  N  S  G    140
        TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAACAACAAATGGTTAACTCTGGT
    361 ---------+---------+---------+---------+---------+---------+  420
        ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACCAATTGAGACCA

        I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F  I  R  A    160
        ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT
    421 ---------+---------+---------+---------+---------+---------+  480
        TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

        S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F  Q  S  A    180
        TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT
    481 ---------+---------+---------+---------+---------+---------+  540
        AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

        K  L  A  D  P  G  A  N  P  H  Q  A  S  P  V  I  N  V  I  I    200
        AAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTAACGTTATTATT
    541 ---------+---------+---------+---------+---------+---------+  600
        TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAATTGCAATAATAA

        P  E  G  A  G  Y  N  N  T  L  D  H  G  L  C  T  A  F  E  E    220
        CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTCGAAGAA
    601 ---------+---------+---------+---------+---------+---------+  660
        GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT
```

```
       S  E  L  G  D  D  V  E  A  N  F  T  A  V  F  A  P  P  I  R     240
       TCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTCGCTCCACCAATTAGA
 661   ---------+---------+---------+---------+---------+---------+   720
       AGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT

       A  R  L  E  A  H  L  P  G  V  N  L  T  D  E  D  V  V  N  L     260
       GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACGTTGTTAACTTG
 721   ---------+---------+---------+---------+---------+---------+   780
       CGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGCAACAATTGAAC

       M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  Q  L  S  P     280
       ATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTCAATTGTCTCCA
 781   ---------+---------+---------+---------+---------+---------+   840
       TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT

       F  C  D  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  S  L  G     300
       TTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT
 841   ---------+---------+---------+---------+---------+---------+   900
       AAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA

       K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  G  F  V     320
       AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT
 901   ---------+---------+---------+---------+---------+---------+   960
       TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCAA

       N  E  L  I  A  R  L  T  H  S  P  V  Q  D  H  T  S  T  N  H     340
       AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC
 961   ---------+---------+---------+---------+---------+---------+  1020
       TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

       T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A  D  F  S     360
       ACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACGCTGACTTCTCT
1021   ---------+---------+---------+---------+---------+---------+  1080
       TGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

       H  D  N  T  M  V  S  I  F  F  A  L  G  L  Y  N  G  T  K  P     380
       CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA
1081   ---------+---------+---------+---------+---------+---------+  1140
       GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTCGGT

       L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A  S  W  T     400
       TTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTGCTTCTTGGACT
1141   ---------+---------+---------+---------+---------+---------+  1200
       AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

       V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  E  A  E  K  E  P     420
       GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA
1201   ---------+---------+---------+---------+---------+---------+  1260
       CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT

       L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  G  V  D  K     440
       TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGGTGTTGACAAG
1261   ---------+---------+---------+---------+---------+---------+  1320
       AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACCACAACTGTTC
```

```
     L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G    460
     TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 ---------+---------+---------+---------+---------+---------+ 1380
     AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

     N  W  E  E  C  F  A  *    467
     AACTGGGAAGAATGTTTCGCTTAA
1381 ---------+---------+---- 1404
     TTGACCCTTCTTACAAAGCGAATT
```

<u>Figure 9</u>

```
     M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T    20
     ATGGGGGTTTTCGTCGTTCTATTATCTATCGCGACTCTGTTCGGCAGCACATCGGGCACT
   1 ---------+---------+---------+---------+---------+---------+ 60
     TACCCCCAAAAGCAGCAAGATAATAGATAGCGCTGAGACAAGCCGTCGTGTAGCCCGTGA

     A  L  G  P  R  G  N  H  S  K  S  C  D  T  V  D  L  G  Y  Q    40
     GCGCTGGGCCCCCGTGGAAATCACTCCAAGTCCTGCGATACGGTAGACCTAGGGTACCAG
  61 ---------+---------+---------+---------+---------+---------+ 120
     CGCGACCCGGGGGCACCTTTAGTGAGGTTCAGGACGCTATGCCATCTGGATCCCATGGTC

     C  S  P  A  T  S  H  L  W  G  T  Y  S  P  Y  F  S  L  E  D    60
     TGCTCCCCTGCGACTTCTCATCTATGGGGCACGTACTCGCCATaCTTTTCGCTCGAGGAC
 121 ---------+---------+---------+---------+---------+---------+ 180
     ACGAGGGGACGCTGAAGAGTAGATACCCCGtgCATGAGCGGTAtGAAAAGCGAGCTCCTG

     E  L  S  V  S  S  K  L  P  K  D  C  R  I  T  L  V  Q  V  L    80
     GAGCTGTCCGTGTCGAGTAAGCTTCCCAAGGATTGCCGGATCACCTTGGTACAGGTGCTA
 181 ---------+---------+---------+---------+---------+---------+ 240
     CTCGACAGGCACAGCTCATTCGAAGGGTTCCTAACGGCCTAGTGGAACCATGTCCACGAT

     S  R  H  G  A  R  Y  P  T  S  S  K  S  K  K  Y  K  K  L  I    100
     TCGCGCCATGGAGCGCGGTACCCAACCAGCTCCAAGAGCAAAAAGTATAAGAAGCTTaTt
 241 ---------+---------+---------+---------+---------+---------+ 300
     AGCGCGGTACCTCGCGCCATGGGTTGGTCGAGGTTCTCGTTTTTCATATTCTTCGAAtAa

     T  A  I  Q  A  N  A  T  D  F  K  G  K  Y  A  F  L  K  T  Y    120
     ACGGCGATCCAGGCCAATGCCACCGACTTCAAGGGCAAGTacGCCTTTTTGAAGACGTAC
 301 ---------+---------+---------+---------+---------+---------+ 360
     TGCCGCTAGGTCCGGTTACGGTGGCTGAAGTTCCCGTTCAtgCGGAAAAACTTCTGCATG

     N  Y  T  L  G  A  D  D  L  T  P  F  G  E  Q  Q  L  V  N  S    140
     AACTATACTCTGGGTGCGGATGACCTCACTCCCTTTGGGGAGCAGCAGCTGGTGAACTCG
 361 ---------+---------+---------+---------+---------+---------+ 420
     TTGATATGAGACCCACGCCTACTGGAGTGAGGGAAACCCCTCGTCGTCGACCACTTGAGC

     G  I  K  F  Y  Q  R  Y  K  A  L  A  R  S  V  V  P  F  I  R    160
     GGCATCAAGTTCTACCAGAGGTACAAGGCTCTGGCGCGCAGTGTGGTGCCGTTTATTCGC
 421 ---------+---------+---------+---------+---------+---------+ 480
     CCGTAGTTCAAGATGGTCTCCATGTTCCGAGACCGCGCGTCACACCACGGCAAATAAGCG

     A  S  G  S  D  R  V  I  A  S  G  E  K  F  I  E  G  F  Q  Q    180
     GCCTCAGGCTCGGACCGGGTTATTGCTTCGGGAGAGAAGTTCATCGAGGGGTTCCAGCAG
 481 ---------+---------+---------+---------+---------+---------+ 540
     CGGAGTCCGAGCCTGGCCCAATAACGAAGCCCTCTCTTCAAGTAGCTCCCCAAGGTCGTC

     A  K  L  A  D  P  G  A  T  N  R  A  A  P  A  I  S  V  I  I    200
     GCGAAGCTGGCTGATCCTGGCGCGACGAACCGCGCCGCTCCGGCGATTAGTGTGATTATT
 541 ---------+---------+---------+---------+---------+---------+ 600
     CGCTTCGACCGACTAGGACCGCGCTGCTTGGCGCGGCGAGGCCGCTAATCACACTAATAA

     P  E  S  E  T  F  N  N  T  L  D  H  G  V  C  T  K  F  E  A    220
     CCGGAGAGCGAGACGTTCAACAATACGCTGGACCACGGTGTGTGCACGAAGTTTGAGGCG
 601 ---------+---------+---------+---------+---------+---------+ 660
     GGCCTCTCGCTCTGCAAGTTGTTATGCGACCTGGTGCCACACACGTGCTTCAAACTCCGC
```

```
      S  Q  L  G  D  E  V  A  A  N  F  T  A  L  F  A  P  D  I  R   240
      AGTCAGCTGGGAGATGAGGTTGCGGCCAATTTCACTGCGCTCTTTGCACCCGACATCCGA
 661  ---------+---------+---------+---------+---------+---------+ 720
      TCAGTCGACCCTCTACTCCAACGCCGGTTAAAGTGACGCGAGAAACGTGGGCTGTAGGCT

      A  R  L  E  K  H  L  P  G  V  T  L  T  D  E  D  V  V  S  L   260
      GCTCGCctCGAGAAGCATCTTCCTGGCGTGACGCTGACAGACGAGGACGTTGTCAGTCTA
 721  ---------+---------+---------+---------+---------+---------+ 780
      CGAGCGGaGCTCTTCGTAGAAGGACCGCACTGCGACTGTCTGCTCCTGCAACAGTCAGAT

      M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  S  Q  L  S  P   280
      ATGGACATGTGTcCGTTTGATACGGTAGCGCGCACCAGCGACGCAAGTCAGCTGTCACCG
 781  ---------+---------+---------+---------+---------+---------+ 840
      TACCTGTACACAgGCAAACTATGCCATCGCGCGTGGTCGCTGCGTTCAGTCGACAGTGGC

      F  C  Q  L  F  T  H  N  E  W  K  K  Y  D  Y  L  Q  S  L  G   300
      TTCTGTCAACTCTTCACTCACAATGAGTGGAAGAAGTACgACTACCTTCAGTCCTTGGGC
 841  ---------+---------+---------+---------+---------+---------+ 900
      AAGACAGTTGAGAAGTGAGTGTTACTCACCTTCTTCATGcTGATGGAAGTCAGGAACCCG

      K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  I  G  F  T   320
      AAGTACTACGGCTACGGCGCAGGCAACCCTCTGGGACCGGCTCAGGGGATAGGGTTCACC
 901  ---------+---------+---------+---------+---------+---------+ 960
      TTCATGATGCCGATGCCGCGTCCGTTGGGAGACCCTGGCCGAGTCCCCTATCCCAAGTGG

      N  E  L  I  A  R  L  T  R  S  P  V  Q  D  H  T  S  T  N  S   340
      AACGAGCTGATTGCCCGGTTGACgCGTTCGCCAGTGCAGGACCACACCAGCACTAACTCG
 961  ---------+---------+---------+---------+---------+---------+ 1020
      TTGCTCGACTAACGGGCCAACTGcGCAAGCGGTCACGTCCTGGTGTGGTCGTGATTGAGC

      T  L  V  S  N  P  A  T  F  P  L  N  A  T  M  Y  V  D  F  S   360
      ACTCTAGTCTCCAACCCGGCCACCTTCCCGTTGAACGCTACCATGTACGTCGACTTTTCA
1021  ---------+---------+---------+---------+---------+---------+ 1080
      TGAGATCAGAGGTTGGGCCGGTGGAAGGGCAACTTGCGATGGTACATGCAGCTGAAAAGT

      H  D  N  S  M  V  S  I  F  F  A  L  G  L  Y  N  G  T  E  P   380
      CACGACAACAGCATGGTTTCCATCTTCTTTGCATTGGGCCTGTACAACGGCACTGAACCC
1081  ---------+---------+---------+---------+---------+---------+ 1140
      GTGCTGTTGTCGTACCAAAGGTAGAAGAAACGTAACCCGGACATGTTGCCGTGACTTGGG

      L  S  R  T  S  V  E  S  A  K  E  L  D  G  Y  S  A  S  W  V   400
      TTGTCCCGGACCTCGGTGGAAAGCGCCAAGGAATTGGATGGGTATTCTGCATCCTGGGTG
1141  ---------+---------+---------+---------+---------+---------+ 1200
      AACAGGGCCTGGAGCCACCTTTCGCGGTTCCTTAACCTACCCATAAGACGTAGGACCCAC

      V  P  F  G  A  R  A  Y  F  E  T  M  Q  C  K  S  E  K  E  P   420
      GTGCCTTTCGGCGCGCGAGCCTACTTCGAGACGATGCAATGCAAGTCGGAAAAGGAGCCT
1201  ---------+---------+---------+---------+---------+---------+ 1260
      CACGGAAAGCCGCGCGCTCGGATGAAGCTCTGCTACGTTACGTTCAGCCTTTTCCTCGGA

      L  V  R  A  L  I  N  D  R  V  V  P  L  H  G  C  D  V  D  K   440
      CTTGTTCGCGCTTTGATTAATGACCGGGTTGTGCCACTGCATGGCTGCGATGTGGACAAG
1261  ---------+---------+---------+---------+---------+---------+ 1320
      GAACAAGCGCGAAACTAATTACTGGCCCAACACGGTGACGTACCGACGCTACACCTGTTC
```

```
      L  G  R  C  K  L  N  D  F  V  K  G  L  S  W  A  R  S  G  G    460
      CTGGGGCGATGCAAGCTGAATGACTTTGTCAAGGGATTGAGTTGGGCCAGATCTGGGGGC
1321  ---------+---------+---------+---------+---------+---------+ 1380
      GACCCCGCTACGTTCGACTTACTGAAACAGTTCCCTAACTCAACCCGGTCTAGACCCCCG

      N  W  G  E  C  F  S  *    467
      AACTGGGGAGAGTGCTTTAGTTGA
1381  ---------+---------+---- 1404
      TTGACCCCTCTCACGAAATCAACT
```

Figure 10

```
     CP-1
      Eco RI M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T
     TATATGAATTCATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCA
  1  ---------+---------+---------+---------+---------+---------+ 60
     ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT

      S  G  T  A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G
     CATCCGGTACCGCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG
 61  ---------+---------+---------+---------+---------+---------+ 120
     GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC
           CP-2
                 CP-3
      Y  Q  C  F  P  E  I  S  H  L  W  G  Q  Y  S  P  Y  F  S  L
     GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT
121  ---------+---------+---------+---------+---------+---------+ 180
     CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTATGAAGAGAA

      E  D  E  S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q
     TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTC
181  ---------+---------+---------+---------+---------+---------+ 240
     ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG
                         CP-4.7
                               CP-5.7
      V  L  S  R  H  G  A  R  Y  P  T  D  S  K  G  K  K  Y  S  A
     AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTgacTCTAAGggtAAGaagTACTCTG
241  ---------+---------+---------+---------+---------+---------+ 300
     TTCAAAACAGATCTGTGCCACGATCTATGGGTTGActgAGATTCccaTTCttcATGAGAC

      L  I  E  A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K
     CTTTGATTGAAGCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
301  ---------+---------+---------+---------+---------+---------+ 360
     GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT
                                     CP-6
                                           CP-7
      T  Y  N  Y  T  L  G  A  D  D  L  T  P  F  G  E  N  Q  M  V
     AGACTTACAACTACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGG
361  ---------+---------+---------+---------+---------+---------+ 420
     TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC

      N  S  G  I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F
     TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT
421  ---------+---------+---------+---------+---------+---------+ 480
     AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA
                                               CP-8.7
                                                    CP-9
      I  R  A  S  G  S  S  R  V  I  A  S  A  E  K  F  I  E  G  F
     TCATTAGAGCTTCTGGTTCTtctAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT
481  ---------+---------+---------+---------+---------+---------+ 540
     AGTAATCTCGAAGACCAAGAagaTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

      Q  S  A  K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  D
     TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG
541  ---------+---------+---------+---------+---------+---------+ 600
     AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAAC
```

```
                                                            CP-10.7
                                                                CP-11.7
      V  I  I  S  E  A  S  S  Y  N  N  T  L  D  P  G  T  C  T  A
     ACGTTATTATTtctGAcgctTCTtctTACAACAACACTTTGGACccaGGTACTTGTACTG
 601 ---------+---------+---------+---------+---------+---------+ 660
     TGCAATAATAAagaCTgcgaAGGagaATGTTGTTGTGAAACCTGggtCCATGAACATGAC

      F  E  D  S  E  L  A  D  T  V  E  A  N  F  T  A  L  F  A  P
     CTTTCGAAGACTCTGAATTGgctGACactGTTGAAGCTAACTTCACTGCTTTGTTCGCTC
 661 ---------+---------+---------+---------+---------+---------+ 720
     GAAAGCTTCTGAGACTTAACcgaCTGtgaCAACTTCGATTGAAGTGACGAAACAAGCGAG
                                                          CP-12.7

      A  I  R  A  R  L  E  A  D  L  P  G  V  T  L  T  D  T  E  V
     CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACactgaaG
 721 ---------+---------+---------+---------+---------+---------+ 780
     GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGtgacttC

     CP-13.7
      T  Y  L  M  D  M  C  S  F  E  T  V  A  R  T  S  D  A  T  E
     TTactTACTTGATGGACATGTGTtctTTCGAAACTGTTGCTAGAACTTCTGACGCTACTG
 781 ---------+---------+---------+---------+---------+---------+ 840
     AAtgaATGAACTACCTGTACACAagaAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

      L  S  P  F  C  A  L  F  T  H  D  E  W  R  H  Y  D  Y  L  Q
     AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGAcacTACGACTACTTGC
 841 ---------+---------+---------+---------+---------+---------+ 900
     TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTgtgATGCTGATGAACG
         CP-14.7
             CP-15.7
      S  L  K  K  Y  Y  G  H  G  A  G  N  P  L  G  P  T  Q  G  V
     AATCTTTGaagAAGTACTACGGTcacGGTGCTGGTAACCCATTGGGTCCAactCAAGGTG
 901 ---------+---------+---------+---------+---------+---------+ 960
     TTAGAAACttcTTCATGATGCCAgtgCCACGACCATTGGGTAACCCAGGTtgaGTTCCAC

      G  F  A  N  E  L  I  A  R  L  T  R  S  P  V  Q  D  H  T  S
     TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT
 961 ---------+---------+---------+---------+---------+---------+ 1020
     AACCAAAGCGATTGCTTAACTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA
                CP-16
                   CP-17.7
      T  N  H  T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A
     CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG
1021 ---------+---------+---------+---------+---------+---------+ 1080
     GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

      D  F  S  H  D  N  G  I  I  S  I  F  F  A  L  G  L  Y  N  G
     CTGACTTCTCTCACGACAACggtattATTTCTATTTTCTTCGCTTTGGGTTTGTACAACG
1081 ---------+---------+---------+---------+---------+---------+ 1140
     GACTGAAGAGAGTGCTGTTGccataaTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC
                            CP-18.7
                                CP-19.7
      T  A  P  L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  S
     GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTt
1141 ---------+---------+---------+---------+---------+---------+ 1200
     CATGACGAGGTAACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGAa
```

```
      A  W  T  V  P  F  A  S  R  A  Y  V  E  M  M  Q  C  Q  A  E
     ctgctTGGACTGTTCCATTCgcttctAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
1201 ---------+---------+---------+---------+---------+---------+ 1260
     gacgaACCTGACAAGGTAAGcgaagaTCTCGAATGCAACTTTACTACGTTACAGTTCGAC
                                                CP-20
                                                    CP-21
      K  E  P  L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A
     AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC

      V  D  K  L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R
     CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA
1321 ---------+---------+---------+---------+---------+---------+ 1380
     GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
                                                         CP-22
      S  G  G  N  W  A  E  C  F  A  *  Eco RI
     GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA
1381 ---------+---------+---------+---------+------ 1426
     CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT
```

Figure 11

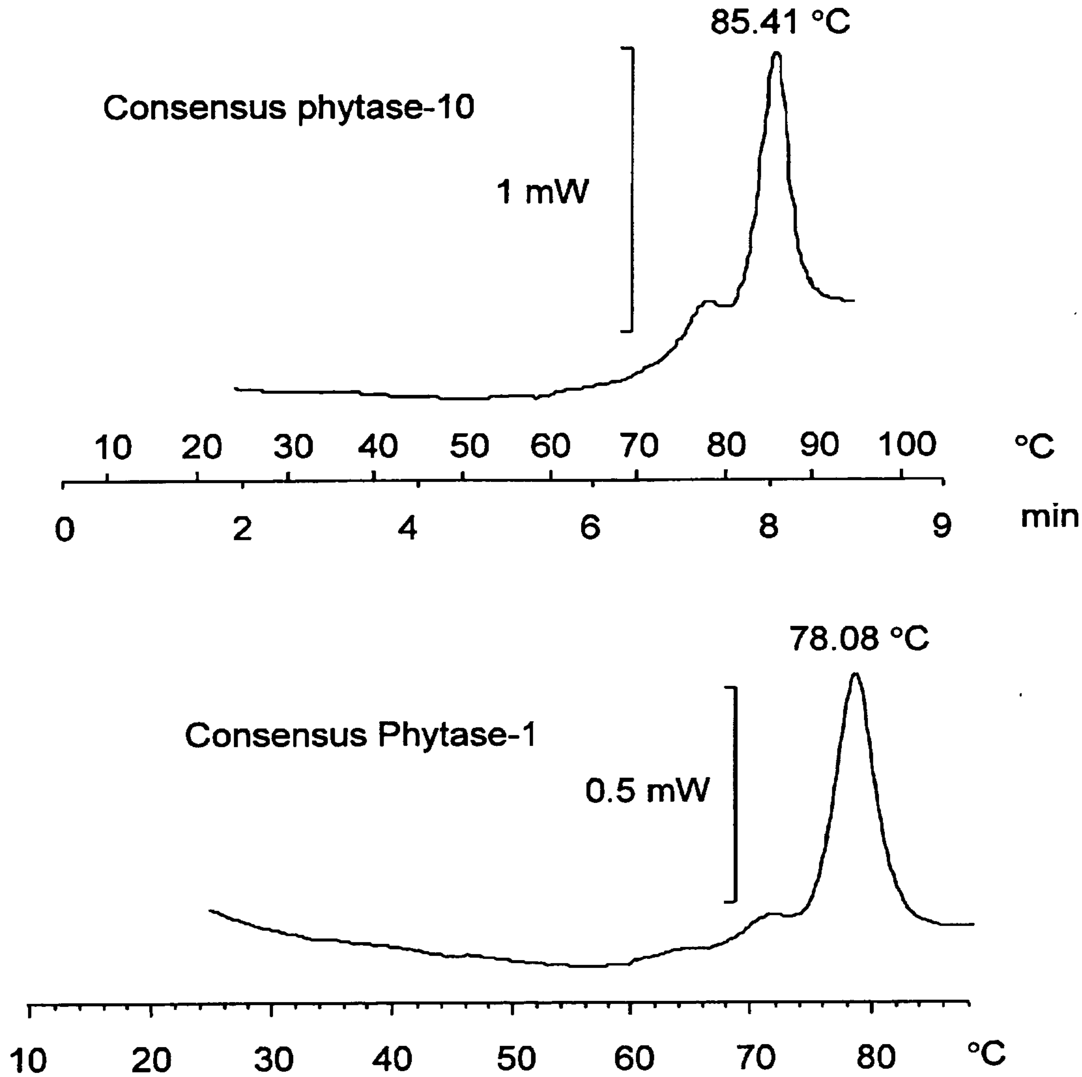
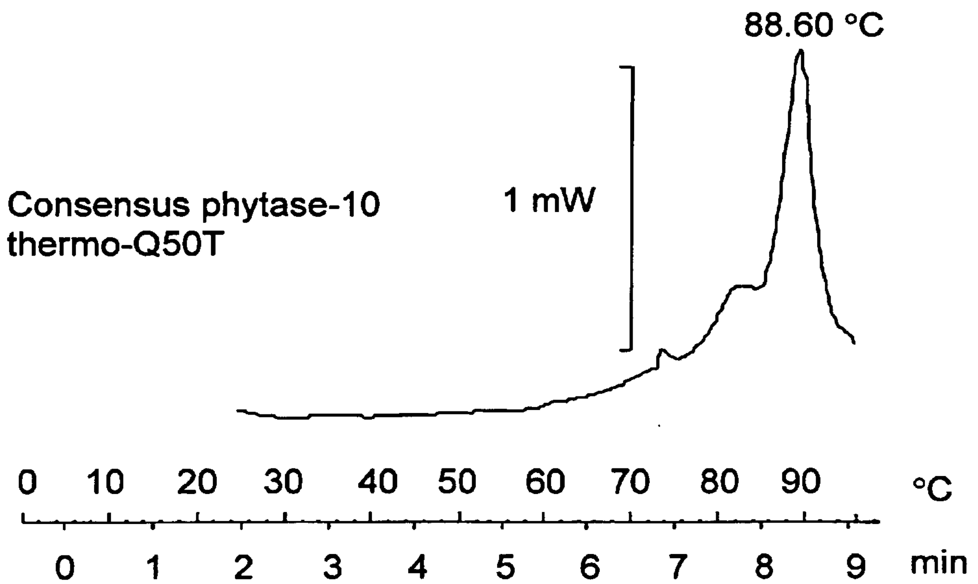

85.41 °C
Consensus phytase-10
1 mW
10 20 30 40 50 60 70 80 90 100 °C
0 2 4 6 8 9 min
78.08 °C
Consensus Phytase-1
0.5 mW
10 20 30 40 50 60 70 80 °C

Figure 12


88.60 °C
Consensus phytase-10
thermo-Q50T
1 mW
0 10 20 30 40 50 60 70 80 90 °C
0 1 2 3 4 5 6 7 8 9 min

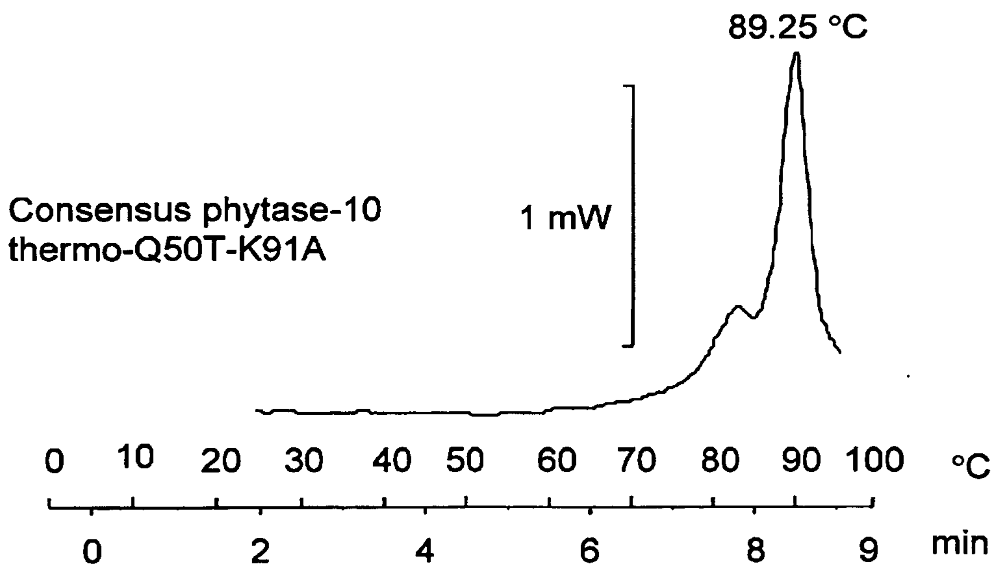
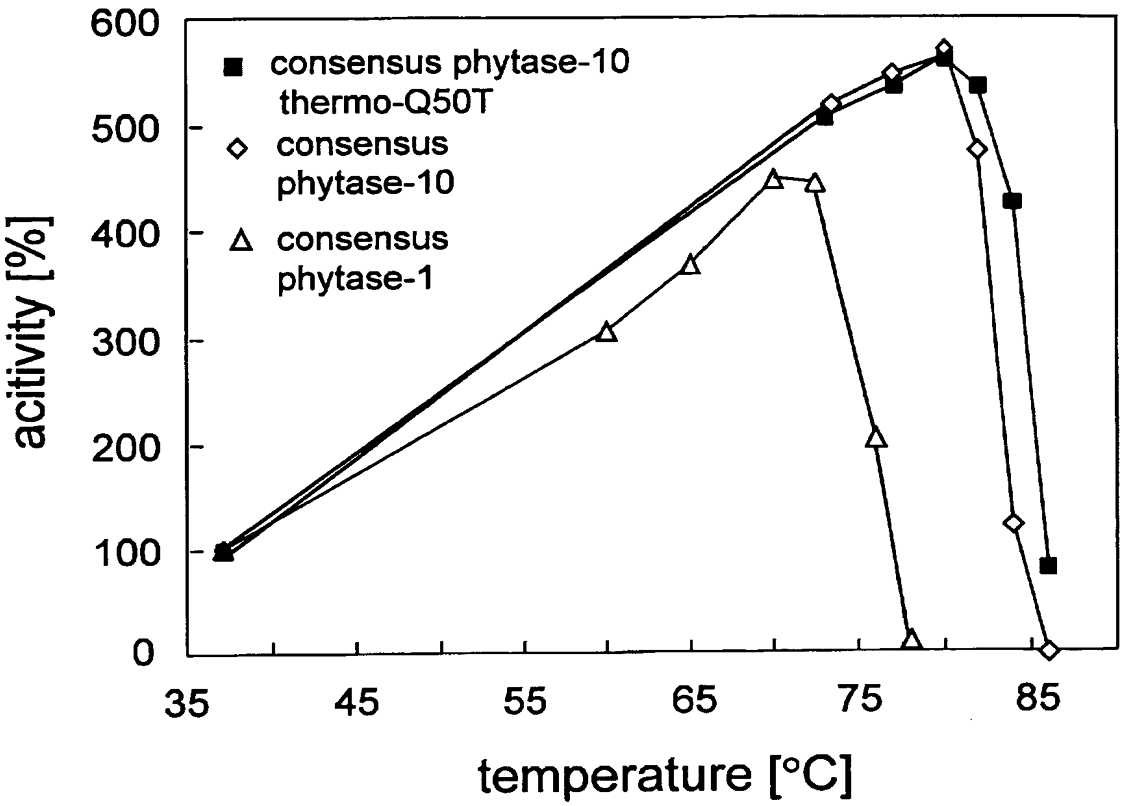

89.25 °C
Consensus phytase-10
thermo-Q50T-K91A
1 mW
0 10 20 30 40 50 60 70 80 90 100 °C
0 2 4 6 8 9 min

Figure 13



Modtaget PD

2. SEP. 1999.

Figure 14

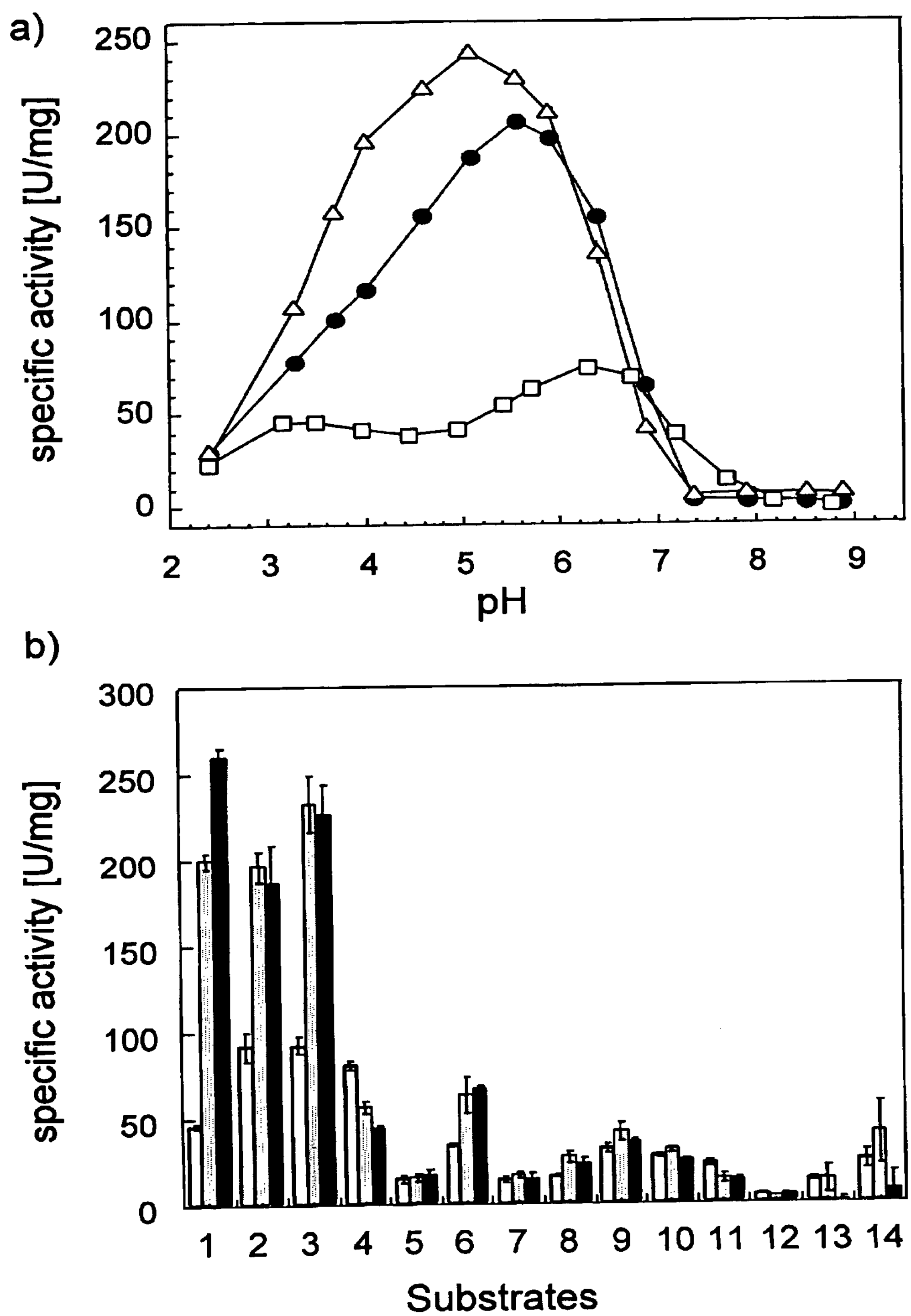
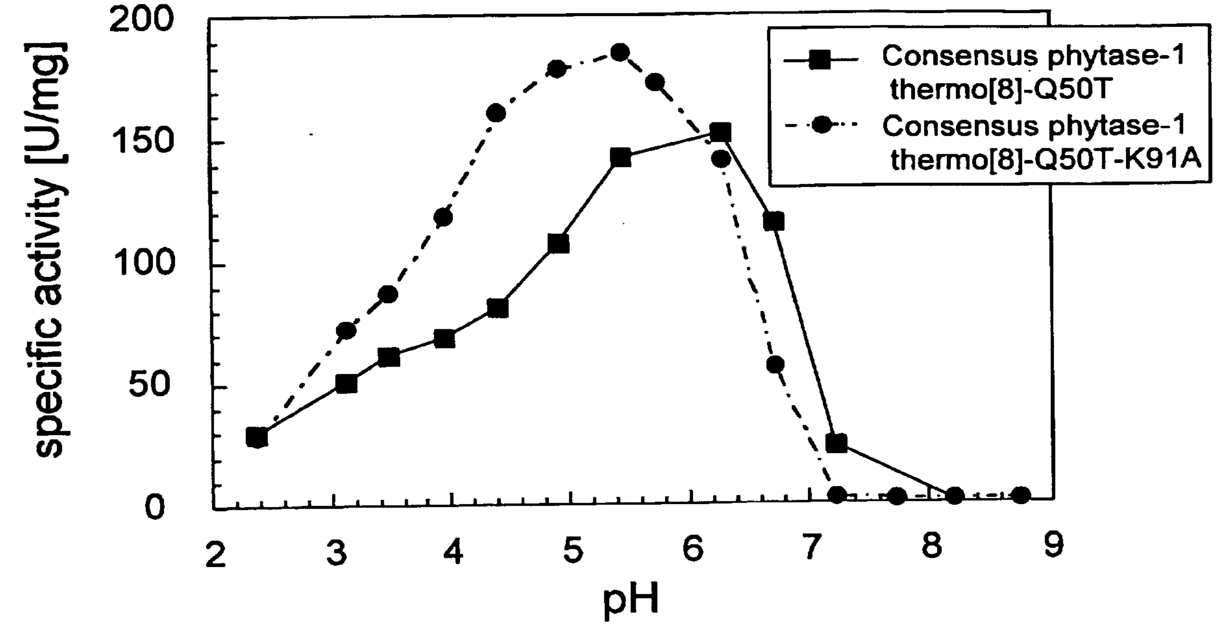

a)
250
200
150
100
50
0
specific activity [U/mg]
2
3
4
5
6
7
8
9
pH
b)
300
250
200
150
100
50
0
specific activity [U/mg]
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Substrates

Figure 15


specific activity [U/mg]
200
150
100
50
0
2
3
4
5
6
7
8
9
pH
Consensus phytase-1 thermo[8]-Q50T
Consensus phytase-1 thermo[8]-Q50T-K91A

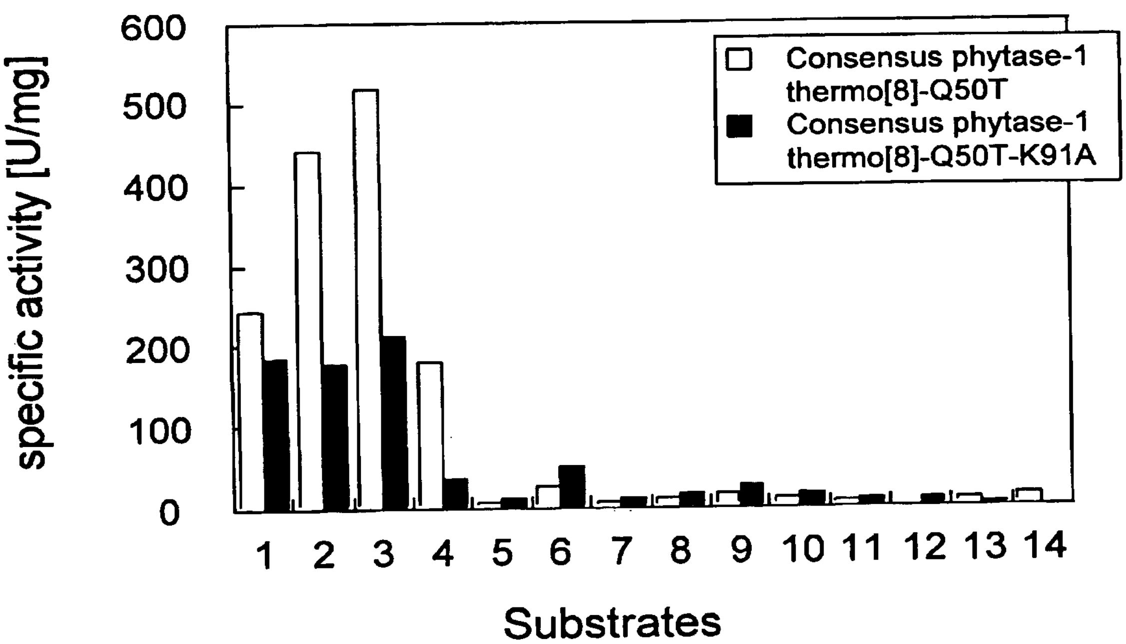
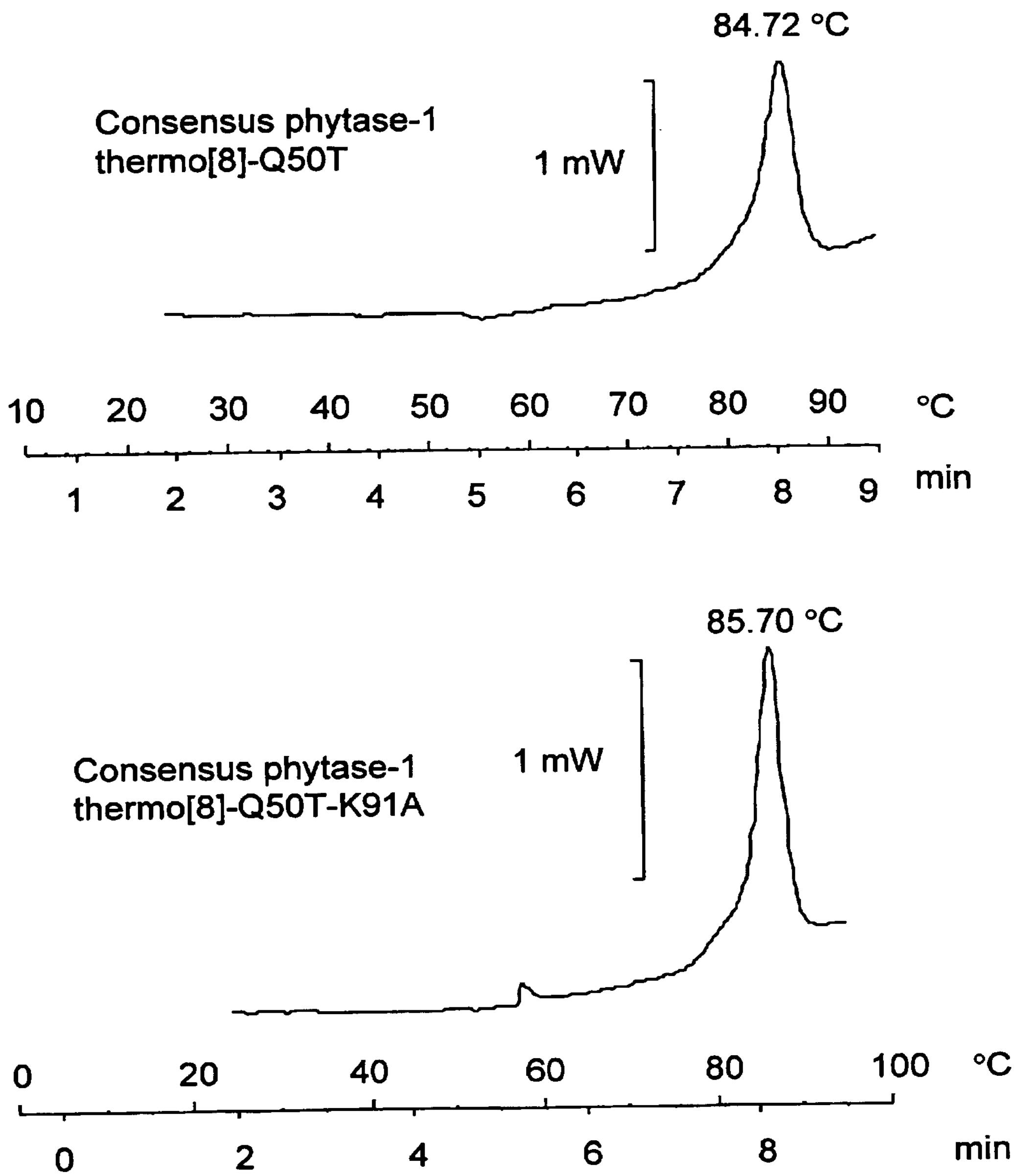

specific activity [U/mg]
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Substrates
Consensus phytase-1 thermo[8]-Q50T
Consensus phytase-1 thermo[8]-Q50T-K91A

Figure 16



Modtaget PD

21 SEP. 1999

Figure 17

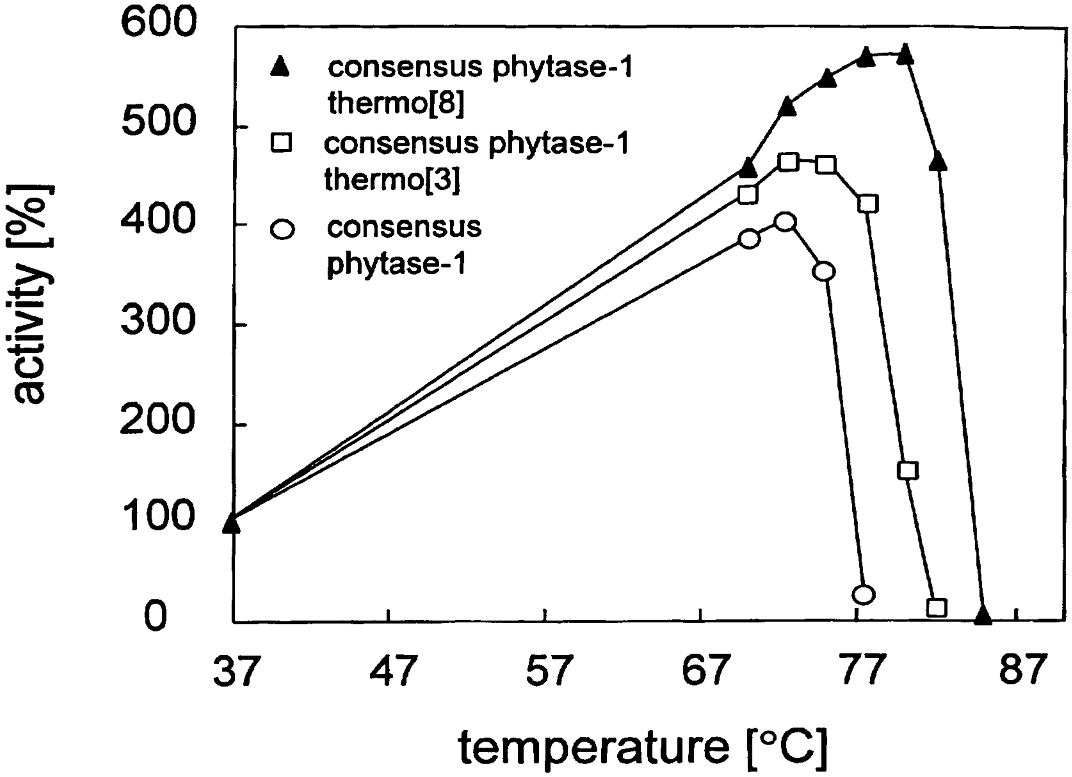

600
500
400
300
200
100
0
activity [%]
consensus phytase-1 thermo[8]
consensus phytase-1 thermo[3]
consensus phytase-1
37
47
57
67
77
87
temperature [°C]

Figure 18

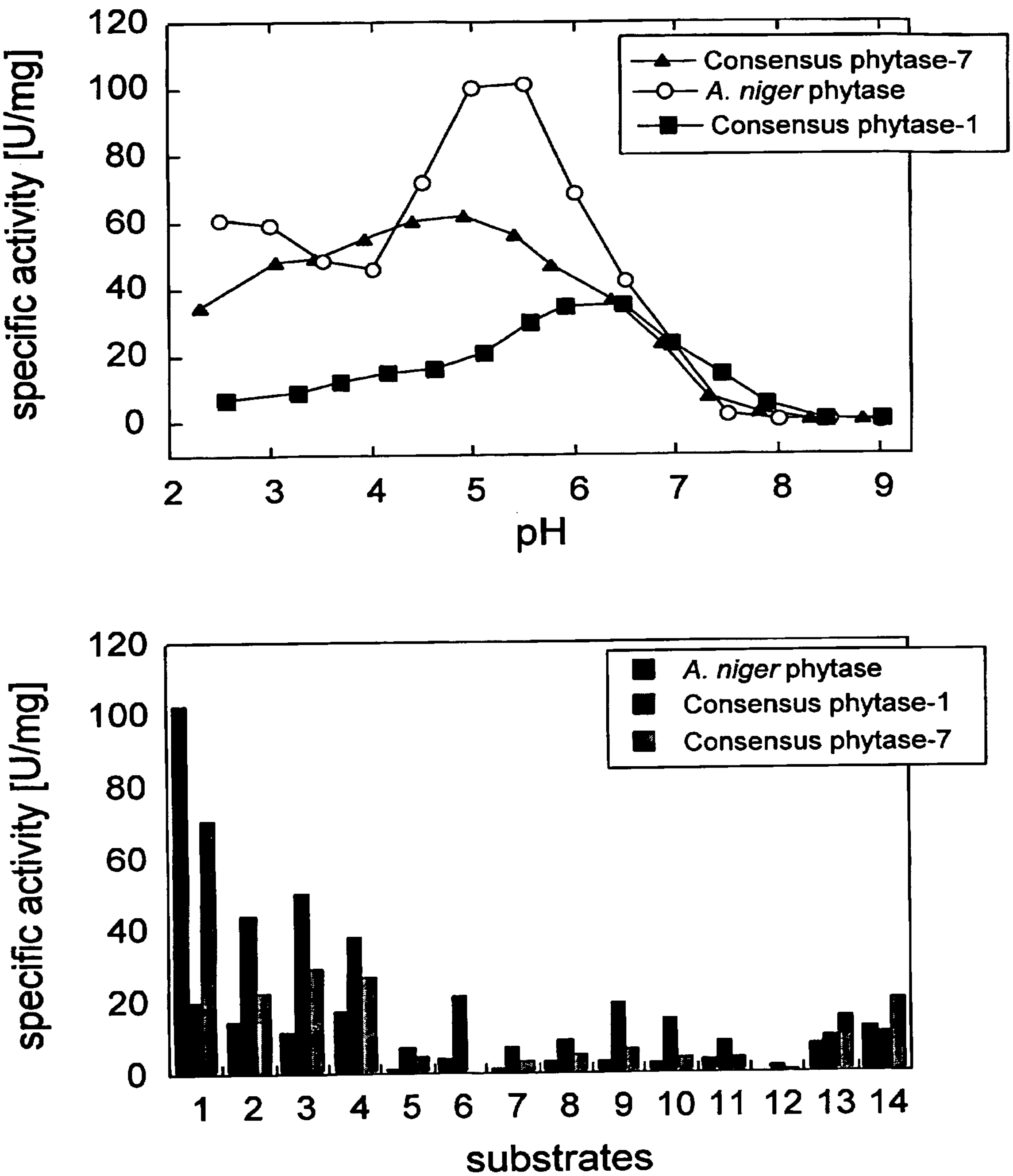
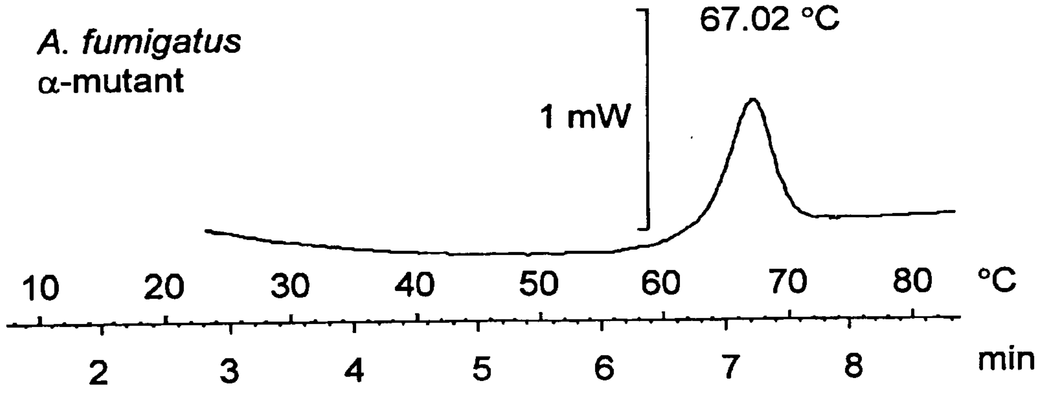

specific activity [U/mg]
120
100
80
60
40
20
0
2
3
4
5
6
7
8
9
pH
Consensus phytase-7
A. niger phytase
Consensus phytase-1
specific activity [U/mg]
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
substrates
A. niger phytase
Consensus phytase-1
Consensus phytase-7

Figure 19


A. fumigatus
α-mutant
1 mW
67.02 °C
10 20 30 40 50 60 70 80 °C
2 3 4 5 6 7 8 min

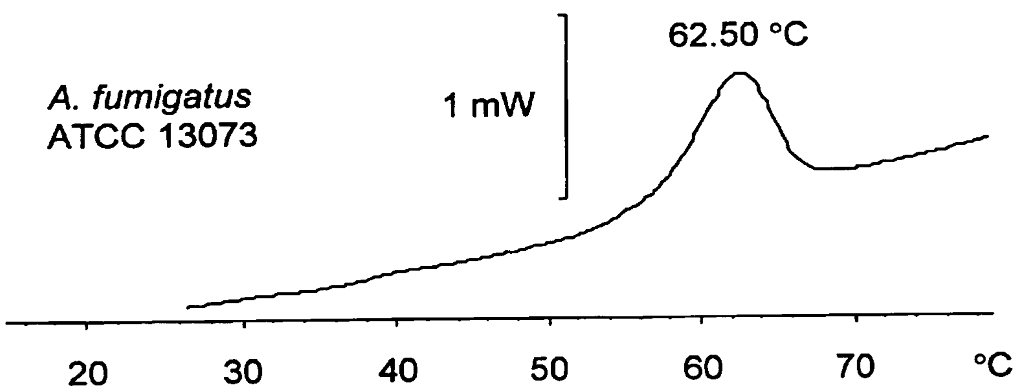

A. fumigatus
ATCC 13073
1 mW
62.50 °C
20 30 40 50 60 70 °C

Figure 20

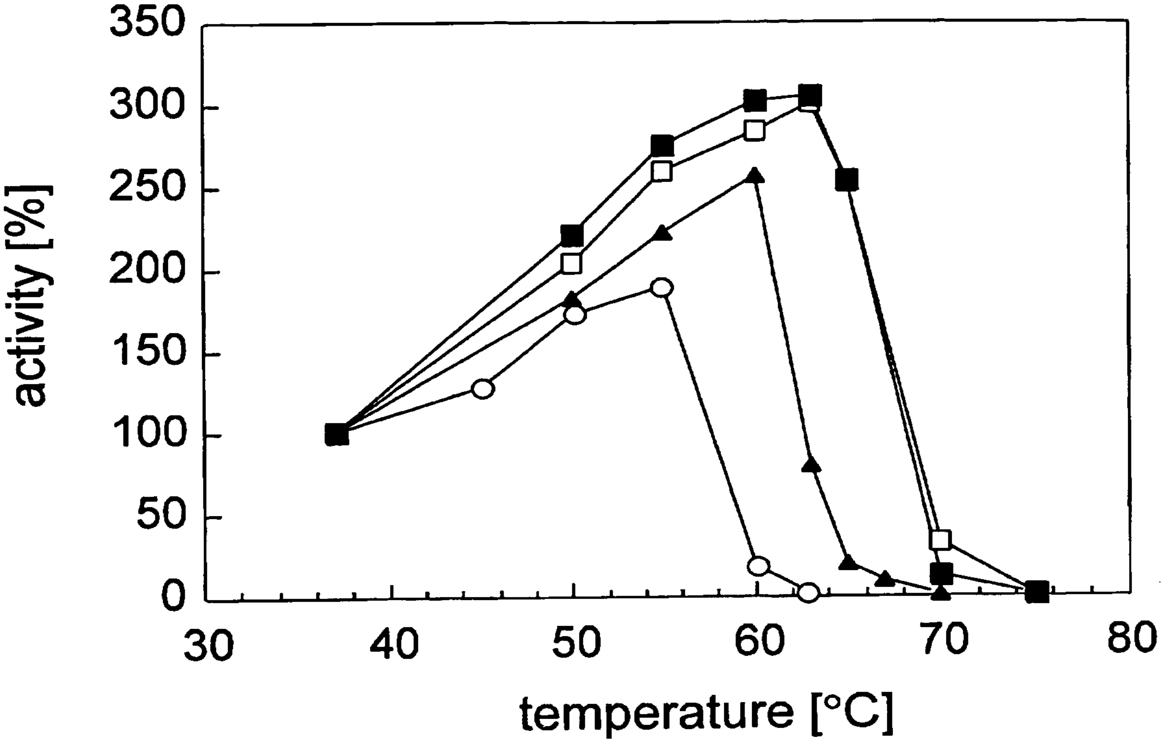

350
300
250
200
150
100
50
0
30
40
50
60
70
80
activity [%]
temperature [°C]

Figure 21

1 MGVFVVLLSI ATLFGSTSGT ALGPRGNSHS CDTVDGGYQC FPEISSNWSP

51 YSPYFSLADE SAISPDVPKG CRVTFVQVLQ RHGARFPTSG AATRISALIE

101 AIQKNATAFK GKYAFLKTYN YTLGADDLVP FGANQSSQAG IKFYRRYKAL

151 ARKIVPFIRA SGSDRVIDSA TNWIEGFQSA KLADPGANPH QASPVINVII

201 PEGAGYNNTL DHGLCTAFEE SELGDDVEAN FTAVFAPPIR ARLEAHLPGV

251 NLTDEDVVNL MDMCPFDTVA RTSDATELSP FCDLFTHDEW IQYDYLGDLD

301 KYYGTGAGNP LGPAQGVGFV NELIARLTHS PVQDHTSTNH TLDSNPATFP

351 LNATLYADFS HDNTMVAIFF ALGLYNGTKP LSTTSVESIE ETDGYSASWL

401 VPFSARMYVE MMQCEAEKEP LVRVLVNDRV VPLHGCGVDK LGRCKRDDFV

451 EGLSFARSGG NWEECFA